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THE INDUCED SUSCEPTIBILITY OF THE GUINEA-PIG TO THE TOXIC ACTION OF THE BLOOD SERUM OF THE HORSE.[1]

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The tests and experiments on which this paper is based were commenced in April, 1903, in connection with the routine of diphtheria antitoxin production. It was customary at that time to inject samples of the serum into animals before putting it on the market in order to guard against any possible contamination with pathogenic bacteria. It was found that when guinea-pigs which had survived an injection with a mixture of diphtheria toxin and antitoxin were used for this purpose, they frequently died within a few hours. For guinea-pigs that had not been used before, the injection was nearly always harmless. During the past year there has been available a considerable material which could be used to extend these observations. This material, together with the data of the earlier tests, was put at my disposal by the director of the laboratory, Dr. Theobald Smith. The preliminary observations were made by him, and I gratefully acknowledge my indebtedness to him for valuable advice throughout the course of my own work in this field.

The phenomena with which this paper concerns itself belong to a class some members of which have long been known, and to which a number of examples have been added within recent years and months. Known to the French as "Anaphylaxis," to the Germans as "Ueber-emfindlichkeit" and to the English as "Hypersensitiveness," or more recently "Supersensitiveness," the class has certain reactions in common. These depend on the fact that certain substances acting on animals continuously or repeatedly, and in suitable dose, render the animal after a time, not immune, but more than normally susceptible to their further toxic action. The substances are unknown in the chemical sense. They are constantly associated with certain mixtures of albuminous substances, bacteria, or bacterial products and are recognized biologically by their specific reaction. An example generally known is the abnormal reaction developed

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when an animal infected with tubercle bacilli is injected with the products of growth of the tubercle bacillus. Blood serum contains a substance, or substances, which under suitable conditions develop a reaction of this character. A number of years ago it was known that the blood serum of one animal was frequently toxic for animals of another species when administered by injection directly into the circulation, and the pathological effects of such toxic action were studied. Flexner(1) (1894) in the report of such a study says, "On the contrary, I found that animals that had withstood one dose of dog's serum would succumb to a second dose given after the lapse of some days, or weeks, even when this dose was sublethal for a control animal." This isolated observation was not developed further.

Richet is credited with an observation of similar import on eel serum, at about the same time. The blood serum of the horse is not toxic for the guinea-pig in the accepted sense of the word, and this may be the reason that the reaction with which we have to deal was not noticed earlier.

Acknowledging a verbal communication from Professor Smith to Professor Ehrlich in regard to the phenomenon, Otto(2), working under the latter, formally drew attention to the reaction which we are now particularly concerned with. He easily repeated the fundamental observation that while horse serum is not a poison for normal guinea-pigs, it causes sudden death or severe illness in animals treated previously with the toxin-antitoxin mixture. He showed that a period of ten days or over must elapse after the injection of the mixture before the serum becomes an active poison. He showed further that the phenomenon could be developed for the blood serum of animals other than the horse, but that it was essentially a specific reaction. That is, horse serum did not become a poison for a guinea pig previously treated with a mixture of toxin and an antitoxin derived from the goat. He developed the fact that normal serum is as effectual in killing animals as is antitoxic serum. He was able to demonstrate a reaction subsequent to a single small dose of normal serum (1/500 c.c.–1/200 c.c.) as a sensitizing treatment, but this reaction was never so severe as in the case of animals treated with the toxin-antitoxin mixture. That is, the animals when tested became very sick, but none died. He states in a footnote, however, that he was able to develop the maximum reaction by giving repeated small doses of horse serum without diphtheria toxin. That is, the toxin was eliminated as an essential factor in the development of the reaction. The animals that survived the reaction, Otto discovered, were immune to subsequent injections of serum. From this he concluded that the substance which killed was a "haptin" in the Ehrlich sense. The more recently developed facts in regard to the immunity indicate that it depends on a combination of reactions which is without well-known analogy.

Rosenau and Anderson(3) and later Anderson(4) by work begun independently confirmed many of these results. They extended their observations in various directions by testing the influence of heat, antiseptics, precipitants, etc., on the toxic substance, and by showing that the offispring of hypersensitive female guinea-pigs are hypersensitive also.

Both Otto and Rosenau and Anderson state positively that death in this manner is unaccompanied by pathological lesions.

Currie (5) writing of the antitoxin rashes in human beings includes the guinea-

pig reaction in his discussion. He reported no experiments with the guinea-pig, and his explanation of the mechanism of the reaction is more complicated than is necessary to explain the facts known in regard to the course of the reaction in the lower animal. Nicolle(6) working with the closely allied "Phenomenon of Arthus" obtained experimental results comparable to my own, and they will be discussed later. Besredka and Steinhardt(7) have experimented with the immunity which is developed when the hypersensitive animal is treated with a large but not fatal dose of serum. Their results touch my work at one point only, and will be referred to later.

Gay and Southard(8) were the first to point out in writing the fact that the intoxication of the hypersensitive animal is accompanied by very obvious anatomical lesions. These lesions in their gross aspects were clearly described in the earliest notes in our laboratory, but were completely overlooked by the earlier experimenters elsewhere. Gay and Southard have worked them out in great detail and the pathological histology of this intoxication is the sound advance made by their work. These writers also formulated a theory to explain the development of the reaction which I will discuss in some detail after reporting on my own work.

Rosenau and Anderson(9) have more recently published results of observations along the lines previously laid down. The advance which concerns us at present is the fact that the transmission of the hypersensitive condition from mother to offspring is ante-natal in its accomplishment. The milk of the mother as tested by experiments in which the nurses were changed is not an essential factor in the transmission. They are now led to the opinion, opposite to that which they formerly held and to that of Otto, that the toxin injected with the sensitizing dose does not affect the degree of the resulting sensitization. It is probable that their earlier opinion is better supported by experiment.

Vaughan and Wheeler(10) have worked on the hypersensitive reaction to egg-white. They hold that the sensitizing dose induces the formation of an entirely new ferment which first gradually disposes of the original proteid injected and then remains for a long time stored in the cells as a zymogen. This zymogen is capable of being activated by the second injection of proteid and of splitting it into a toxic and a non-toxic portion more rapidly than the toxic portion can be safely disposed of. They are able to separate by a process of chemical mutilation a toxic portion which cannot sensitize the animal, and a non-toxic residue which sensitizes against the whole egg-white. Their case differs in important respects from that of the hypersensitiveness against serum. For instance they are unable to demonstrate any newly-formed substance in the blood of the hypersensitive animal which is capable of passively sensitizing a fresh animal.

Otto(11) in a recent paper, which came to hand after the completion of my work, has demonstrated by passive transfer, a newly-formed antibody in the blood serum of the hypersensitive animal. He believes, as I do, that this antibody is distinct from the horse serum "rest" present in the same serum—the "anaphylactin" of Gay and Southard. He does not, however, distinguish the hypersensitive reaction developed in the fresh animal by the transfer of a small amount (.1 c.c. to 2.5 c.c.) of hypersensitive guinea-pig serum after the two weeks' incubation period, from the immediate reaction (twenty-four hours) that can be developed by the transfer of 10 c.c. to 15 c.c. of the same blood or blood

serum. He was able to demonstrate the antibody in the blood serum of the immune or refractory animal, and on this ground doubts the opinion of Bedreka and Steinhardt, that the immunization in this instance is really a desensitization or exhaustion of the antibody. If he had made the more decisive intracirculatory test to determine the full development of the refractory state, or had injected a considerable surplus of serum over the amount needed to enable the animal to withstand the further subcutaneous or intraperitoneal injection of horse serum, he would probably have been willing to agree to their view in its essential features. Otto further shows that the combination of hypersensitive antibody with horse serum has no power to divert to itself guinea-pig complement when tested by the method of Bordet and Gengou. He holds, on general grounds, that this reaction is the manifestation in the guinea-pig of the "phenomenon of Arthus."

It is my purpose to present as briefly as possible our early and more recent experiments, in so far as they show the early, general observations on serum anaphylaxis. In extending these observations my work has been carried on in large part with a view to answering certain specific questions. These questions may be briefly stated, and for purpose of presentation my report of results will be, in part, grouped around them under the following heads:

I. Are the various methods of inducing the hypersensitive state and of detecting it of equal value in the determination of the nature of the reaction and of the factors involved?

II. Synopsis of early experiments.

III. What are the facts in regard to the transmission of anaphylaxis from mother to offspring, and what light do these facts shed on the problem of direct or active sensitization?

IV. What is the mechanism of the acute hypersensitive reaction? More particularly, is it possible to transfer the hypersensitive condition from animal to animal with the blood or blood serum?

V. What are the facts in regard to the immunity which is developed when a hypersensitive animal is treated with a sublethal dose of horse serum and what is the nature of this immunity? Included with the discussion of this question is the rather detailed description of a type of serum hypersensitiveness unusual in the guinea-pig. The reaction is largely localized in the subcutaneous tissues, and results in necrosis.

VI. A more general discussion of the problem as a whole.

VII. Summary.

I. METHODS OF SENSITIZATION AND TEST.

The quantity of serum given at the second or test injection and the method of its administration are of primary importance in the study of the reaction. The results obtained by different workers and especially the interpretation of these results have been influenced in no small degree by the particular method of test chosen by them. In our early work and in that of Otto, the guinea-pigs were sensitized by treatment with non-fatal toxin-antitoxin mixtures. They were tested after four weeks or later with from three to six cubic centimeters of serum injected subcutaneously. Under the circumstances all of the animals proved hypersensitive and about fifty per cent. of the cases were fatal. The incubation period found to be necessary for a positive result was about two weeks, but very few of the animals were used so soon. As other workers came into the field they felt it desirable to push the work faster, and using this method of injection after incubation of from ten to fourteen days they could not get such consistent results. Rosenau and Anderson adopted the intraperitoneal method; Besredka and Steinhardt developed the intracranial injection. Gay and Southard used injections of serum alone to sensitize, and found that they were unable to kill their animal when testing by the subcutaneous method They adopted the intraperitoneal injection for routine work, and called attention to the very great sensitiveness of the animals to a test injection made directly into the circulation. I have not used the intracranial and intraperitoneal methods, and my statements in regard to them are based on the reports of others. I have recently used the direct injection into the circulation for special purposes. I find that the injection directly into the heart is the simplest procedure. After some practice it is quite certain to succeed, and it can be carried out rapidly. About two cubic centimeters of horse serum can be introduced into a normal guinea-pig of 230 grams weight without causing any symptoms. The method, the accidents incident to it, and the controls necessary, are fully described by Morgenroth (12). My technique differs from his in that I work without an assistant, tying the animal out firmly on a suitable board. I use a smoothly-working glass hypodermic syringe of capacity of two and a half cubic centimeters instead of his canula and detached syringe barrel.

Tested by this method after an incubation period of two weeks the certainly fatal dose of serum for animals sensitized by the toxin-antitoxin mixture is probably about 1/100 c.c.; 1/200 c.c. of serum will cause severe symptoms, and 1/150 c.c. will sometimes kill. Thus in testing for the degree of hypersensitiveness it is possible to inject about two hundred certainly fatal doses. By the intracranial method the certainly fatal dose is about 1/20 c.c. By the intraperitoneal route three cubic centimeters is almost certainly fatal. By the subcutaneous method it is probably impossible to reach the certainly fatal dose because of the impossibility of getting rapid absorption. As five or six cubic centimeters always develop a well-marked reaction, it is probable that from fifteen to twenty cubic centimeters, if absorbed at about the same rate, would be certainly fatal. It is impossible to use such an amount in practice. It is obvious that results obtained by one of these methods cannot be at once applied to the subject in general without most careful consideration of the values involved. In working with animals feebly hypersensitive the subcutaneous method would often show no result, while used on animals thoroughly sensitized differences in degree of reaction would be entirely masked by even 0.1 c.c. given into the circulation. For differences among animals that are all very sensitive the subcutaneous injection is capable of giving the most instructive results, unless the delicate methods are more carefully standardized quantitatively than has so far been done. To make an application of the above considerations to the problem in hand we may consider: (*a*) The incubation period; (*b*) the influence of toxin on serum sensitization.

(*a*) The incubation period is not to be considered as abruptly terminating at a given day. I have made an animal quite sick by the intracardiac injection of two cubic centimeters of serum on the sixth day after a toxin-antitoxin mixture. Those who have used the subcutaneous injection at twelve to fourteen days have not had consistent results, but by about three or four weeks the hypersensitiveness seems to reach its maximum. Holding the animals longer than this does not seem to increase the percentage of fatal cases. These rather meager facts make it appear that the antibody on which the reaction depends is produced gradually from a

time very soon after the sensitizing injection, and that the total effective quantity increases for a period of several weeks. About the sixth day, or perhaps somewhat earlier, it can be detected by the most delicate method.

(*b*) Otto published the results of a considerable number of attempts to sensitize his animals with small doses of serum alone. He found that the animals were made hypersensitive, but not to the same degree as when the serum was mixed with toxin. None died when tested by the subcutaneous method. Gay and Southard had the same experience and in order to accomplish their purpose settled on the intra-peritoneal test injection. Recently Rosenau and Anderson have published a table of results showing that toxin does not increase the sensitizing action of serum when combined with it. In these experiments they used the intraperitoneal test injection. It seems plain that the subcutaneous method of testing is the better suited to decide a point which concerns the maximum of sensitization, and I believe that the earlier opinion of Otto is the better supported by experiment.

Accepting the results of Otto, who showed that the animals which had survived the injection of diphtheria toxin alone were normal in their reaction to horse serum, I have used such animals in two groups of four each to determine what treatment, if any, with serum alone would develop the maximum grade of hypersensitiveness. As the accompanying table shows, I have confirmed Otto's observation which he did not report in detail, that repeated small doses of serum alone could do this.

The treatment with 1/1000 c.c. serum given three times on alternate days, with the injection ten days after the third treatment, was about as efficient as the treatment with the toxin-antitoxin mixture and slightly more efficient than a treatment with 1/2000 c.c. serum on ten successive days, with the test treatment ten days after the last injection. Either method is much more efficient than the single treatment with three cubic centimeters or over, or than the single treatment with 0.03 c.c. to 0.0025 c.c. serum, reported in detail by Otto.

TABLE I.—*Effect of Repeated Small Doses of Serum.*

Guinea-pig Number.	Sensitizing Treatment.	Interval Between Treatments.	Test Treatment.	Result of Test Treatment.	Remarks.
4704	1/2000 c.c. serum, 10 injections in 11 days. Total 1/200 c.c.	10 days after last injection, 21 days after first.	5 c.c. serum subcut.	Slight immediate symptoms; late induration.	Slight induration after first injection with 1/2000 c.c. serum.
4701	do	do	do	Same.	
4658	do	do	do	Same + small late ulcer.	
4689	do	do	do	Dead 2 hours.	
4699	1/1000 c.c. serum, 3 times in 6 days. Total 1/333 c.c.	10 days after last injection, 16 days after first.	do	Dead in night.	Slight induration after 1st injection with 1/1000 c.c. serum.
4722	do	do	do	Dead 4 hours.	Well marked swelling after 1st injection of 1/1000 c.c. serum.
4737	do	do	do	Slightly sick; late ulceration.	
4696	do	do	do	Very sick; late ulceration.	

II. SYNOPSIS OF EARLY EXPERIMENTS.

The records of the laboratory show that thirty-eight guinea-pigs bred from untreated mothers and themselves not treated were injected with doses of from three to five cubic centimeters of normal or antitoxic horse serum. Of these not one was noticeably affected. Examined at the end of twelve and twenty-four hours the subcutaneous tissues often show no palpable œdema or induration. The latter statement does not cover the full number of animals reported on, as such examinations were not always made. We have no contrary case, however. Our results are thus in accord with those of others in showing that practically horse serum is not a toxic substance for the normal guinea-pig. But neither is it an indifferent solution of substances which can be entirely eliminated from the subcutaneous tissues by processes which govern the removal of normal saline solution, for example. In several instances the injection of 1/1000 c.c. or 1/2000 c.c. of serum in two cubic centimeters of normal saline solution has given rise to a well-marked œdema at the end of twenty-four hours. Also, as can be demonstrated by suitable test-tube experiments, horse serum normally contained small amounts of ambocepter active between guinea-pig red blood corpuscles and the complement of guinea-pig serum. Certain of its constituents are probably always removed by complex processes similar in kind to those which prevail in the case of the hypersensitive animal, and the tissues may, under certain circumstances, be mildly injured in the course of their elimination.

Thirty-six animals treated with from two and a half to five cubic centimeters of serum as a first dose were subsequently once or twice injected with similar quantities. Of these thirty-three remained without symptom or lesion. One showed symptoms at the second treatment. One died at the second treatment, and one died at the third injection. The intervals between treatments have varied between thirteen days and four months, and have most frequently been between three and six weeks. The animals that died received their fatal treatment after an interval of sixteen days in each case. These results agree with those obtained elsewhere, and show that a single or repeated large dose of serum may render the animal susceptible to a subsequent similar dose, but that it is a

much less efficient way of inducing a hypersusceptibility. It is quite possible that the fatal results in the two cases may be due to the accidental absorption of serum directly into the circulation through a vessel injured by the injection. Or it may be that there is really a very great difference in the reaction of the individual animals to the first large dose. However that may be, our cases are evidently not comparable to those of Gay and Southard, who found that the large dose always rendered the animal hypersensitive if enough time were allowed to elapse before the test injection. As the intervals they report between the sensitizing and intoxicating injections were as a rule shorter than ours, these differences in result must also be attributed to a difference between the effects of the intraperitoneal and subcutaneous injections. In these experiments they used the intraperitoneal route for both first and second injections, and in this way avoided the great binding power which the subcutaneous tissues probably have for the toxic principle here involved.

Twenty-five guinea-pigs which had survived the treatment with a mixture of diphtheria toxin and antitoxin were injected with large doses of horse serum. In each instance in which the dose injected was one cubic centimeter or over the animal was made sick. Fourteen of them died. In our experience as well as in that of Otto and Rosenau and Anderson, it is a law without exception that treatment with a toxin-antitoxin mixture renders the animal susceptible to an acutely acting toxic substance in normal and antitoxic horse serum. The exact amount of serum injected with the mixture, if between 1/100 c.c. and 1/500 c.c., and the exact interval between the injections, if between two weeks and three months, *are indifferent matters* in the development of the reaction. The same may be said of the local lesion caused by the mixture, and of the genealogy of the animal.

The length of time that such an induced susceptibility may persist has not been fully determined so far. I have been able to test thirteen old females with intervals after sensitizing varying from eleven months to two years. Compared with those tested in less than four months after sensitization, these animals gave less reaction. Two of them, at eleven and sixteen months, respectively injected with five cubic centimeters of serum gave no reaction.

Three animals of the series died, one after an interval of twenty-two months between sensitizing and test injections. The others ranged between these extremes. The reaction is thus a slowly disappearing one which may probably in individual instances persist throughout the life of the animal.

III. HYPERSENSITIVENESS TRANSMITTED FROM MOTHER TO OFFSPRING.

Our experience in injecting large doses of serum (from three to six cubic centimeters) subcutaneously into guinea-pigs bred from mothers that had been treated with the toxin-antitoxin mixture or with horse serum alone is shown in the following table.

TABLE II.—*Transmitted Hypersensitiveness.*

Treatment of Mother.	Number of Mothers.	Number of Offspring.	Result.
Toxin + Serum, 1/100 c.c. to 1/500 c.c.	27	41	Well 17 Dead 24
Serum only. Large dose, 3 c.c. to 5 c.c.	4	6	Well 2 Symptoms 2 Dead 2

The fact that the increased susceptibility generated in a female guinea-pig by treatment with a sublethal toxin-antitoxin mixture is transmitted to her offspring as first published by Anderson, is clearly shown. It is also seen that treating the mother with a single large dose of horse serum renders the offspring hypersensitive. Gay and Southard found that the offspring of their guinea-pigs sensitized with serum alone were hypersensitive in several instances But in our experience only a percentage of the animals bred from hypersensitive mothers are abnormal in their reaction. It might be supposed that the mothers of those young which do not react are not themselves hypersensitive. We were able to test this in one case and found that the mother still gave a moderate reaction, although her offspring at the time of test gave none. Furthermore, several females have given birth to individuals of each class, the normal and the hypersensitive. In one case an entire litter (four animals) of one female was tested on the same day with the same

dose of serum (six cubic centimeters subcutaneously). Two of them died within fifteen minutes, the other two gave no reaction. It is obvious that if the statement of Rosenau and Anderson, that the hypersensitive state is always transmitted from the mother to offspring (and by inference to all of them), be essentially true, the law must be limited in its application by certain conditions. To reason from the published experiments of the last mentioned authors, from our own experience, and from analogy with the other cases of transmitted immunity reactions, it seems that hypersensitiveness is by nature transmissible. But the mother probably transmits less effectively if her own initial sensitiveness is low and if the elapsed time between her sensitizing treatment and the birth of the offspring in question is long. The young animals seem to lose their sensitiveness with some rapidity, as they increase in age and size, and it is probable that the individual variation in the rate of this loss is considerable. It must be so if it is to account for the extreme difference between animals of the same litter tested on the same day. Alternatively one could assume an individual difference due to the influence of a normal father, but this would be without known analogy, and could only be justified by prolonged experiment.

The type of reaction obtained in these guinea-pigs which have acquired their increased susceptibility from the mother is interesting and calls for explanation. The animals rendered hypersensitive by treatment with the toxin-antitoxin mixture when treated with a subsequent injection of serum usually begin to show symptoms in about half an hour. Those that die usually do so in from two to four hours. Those that recover are ordinarily most ill at about four hours after the injection. From this time they recover rather rapidly, and are to all appearances well in from six to twelve hours. Occasionally death is delayed twelve hours and complete recovery to twenty-four hours. The animals tested for a transmitted susceptibility have reacted quite differently. Those that have proved hypersensitive have usually died in from fifteen to thirty minutes after injection. Death has occurred in five minutes. In two instances out of twenty-four death took place at the end of two hours; in one instance, in the night after some hours. The animals

that do not die show almost no reaction. They frequently brush the ears and nose with the forefeet and have a staring coat for half an hour, more or less. I have not seen a case in which an animal bred hypersensitive became severely sick and recovered. Rosenau and Anderson publish protocols which do not bear out this experience, but as they have used the intraperitoneal injection altogether the results are not strictly comparable. This sharp distinction between the reaction given by different young animals, extending as it does even to individuals of the same litter, together with the more rapid reaction given by the hypersensitive offspring, cannot perhaps be clearly explained by facts definitely proven at the present writing. Several factors may be mentioned as probably influencing the results. The young animal may be more easily injured by the ultimate toxic substance when it has the capacity to form or assimilate it. Absorption from the subcutaneous tissues in the young animal in so far as it depends on physical conditions, is probably more rapid than in the older one. The subcutaneous tissues of the animal sensitized by direct injection have probably been greatly influenced by the treatment, and in such a way that there is a local hypersensitiveness induced which leads to a local specific absorption of the toxic substance, tending to protect to a degree the cells of more vital organs. This will be rather definitely developed later. Such a local reaction may be less easily transferred from mother to offspring than a more general one depending on the conditions in the blood.

The fact that there is in connection with the phenomenon of serum hypersensitiveness a definite transmission of the susceptibility to the reaction from the mother, to her offspring, is at the present time very strong evidence for the proposition that the sensitizing injection causes the formation of an anti-body with which the second or test injection reacts.

IV. PASSIVE TRANSFER OF THE HYPERSENSITIVE CONDITION.

Gay and Southard attempted to determine the mechanism of the hypersensitive reaction. Their essential experiments from this point of view may be briefly restated. They found that the serum of hypersensitive guinea-pigs mixed with horse serum, incubated

TABLE III.—*Results of the Passive Transfer of Hypersensitiveness.*

Normal Guinea-Pig Number.	Sensitizing Treatment.	Interval.	Intoxicating Treatment.	Result.
5038	27–VI–07. Serum of sensitive G. P. Inj. 1.75 c.c.; intracardiac.	1 hr.	27–VI–07. 1 hr. later. Inj. 1 c.c. normal horse serum; intracardiac.	No symptoms or very slight.
5035	27–VI–07. Ser. of sens. G. P. Inj. 3 c.c.; intraperitoneal.	1 d.	28–VI–07. Ser. nor. horse. Inj. 1 c.c; intracardiac. 5 c.c. intraperitoneal.	Very slight symptoms.
5044	28–VI–07. Ser. of sens. G. P. Inj. 5 c.c.; intraperitoneal.	3 d.	1–VII–07. Ser. nor. horse. Inj. 2 c.c.; intracardiac.	No symptoms.
5047	28–VI–07. Ser. of sens. G. P. Inj. 2.5 c.c.; intraperitoneal.	6 d.	4–VII–07. Ser. nor. horse. Inj. 1 c.c.; intracardiac.	Slight symptoms.
5045	28–VI–07. Ser. of sens. G. P. Inj. 1.5 c.c.; intraperitoneal.	24 d.	24–VII–07. Inj. 1.1 c.c. ser. nor. horse; intracardiac.	Dead ½ hour.
5036	23–VI–07. Ser. of sens. G. P. Inj. 2.5 c.c.; intraperitoneal.	1 d.	24–VI–07. Ser. nor. horse. Inj. 6 c.c.; intraperitoneal.	Moderate but definite symptoms.
4975	2–VIII–07. Defibrinated blood of sens. G. P. Inj. 14 c.c.; intraperit. and subcut. 4–VIII–10 a. m. Same. Inj. 5 c.c.; intraperitoneal.	2 d.	4–VIII–07, 12 m. Inj. 1.5 c.c. nor. horse serum; intracardiac.	Severe symptoms; chloroform 4 hrs.; hemorrhagic lesions.
6010	Freshly defibrinated blood of sens. G. P. 8–VIII–07: Inj. 3 c.c.; intraperit. 9–VIII–07: " 6 c.c.; " 10–VIII–07: " 6 c.c.; " Total 15 c.c.	3 d.	11–VIII–07. Inj. 1.25 c.c. nor. horse serum; intracardiac.	Dead 3 minutes.
5079	Freshly defibrinated blood of sens. G. P. 25–VII–07: Inj. 3 c.c.; intraperit. 26–VII–07: " 5 c.c.; " 27–VII–07: " 2 c.c.; " 28–VII–07: " 5 c.c.; " Total 15 c.c.	4 d.	29–VII–07. Inj. 2 c.c. nor. horse serum; intracardiac.	Dead 4 minutes.

TABLE III.—*Results of the Passive Transfer of Hypersensitiveness.*—Concluded.

Normal Guinea-Pig Number.	Sensitizing Treatment.	Interval.	Intoxicating Treatment.	Result.
6017	19–VIII–07. Freshly defibrinated blood of sens. G. P. Inj. 15 c.c.; intraperititoneal and subcutaneous.	1 d.	20–VIII–07. Serum of normal horse. Inj. 1.5 c.c.; intracardiac.	Dead 2 minutes.
6059	11–IX–07. Serum of sens. G. P. Inj. 7.5 c.c.; intraperitoneal.	1 d.	12–IX–07. Nor. horse ser. Inj. 1.5 c.c.; intracardiac.	Very severe symptoms.
6058	12–IX–07. Serum of sens. G. P. heated to 60° ½ hr. Inj. 7.5 c.c.; intraperitoneal.	1 d.	13–IX–07. Nor. horse serum. Inj. 1.5 c.c.; intracardiac.	Moderate symptoms.
	Control Experiments.			
6013	19–VIII–07. Freshly defibrinated blood of normal G. P. Inj. 15 c.c.; intraperitoneal.	1 d.	20–VIII–07. Ser. of nor. horse. Inj. 2 c.c.; intracardiac.	No symptoms.
5082	23–VII–07. *Diph. Toxin* — .18 c.c. Antitoxic serum 1/100 c.c. +. Inj. subcutaneous slight lesion.	6 d.	29–VII–07. Ser. of nor. horse. Inj. 2 c.c.; intracardiac.	Slight but rather definite symptoms.

Note. — All of the fresh guinea-pigs used in the above experiments except No. 4975 weighed from 230 to 250 grm. It is doubtful if larger animals would have reacted as well. The first five animals illustrate the ultimate sensitization by small doses of sensitive serum. The second control experiment was made for the purpose of showing that the cases in which the passive transfer of sensitive serum occupied several days do not reach the beginning of the effective incubation period for direct or active sensitization even when the latter is tested for by the intracardiac method.

and injected into the circulation of normal animals, provoked no reaction. On the basis of two such experiments they apparently drew the conclusion that the serum of the hypersensitive animal does not contain an anti-body for the toxic substance of horse serum. They further showed that 1.5 c.c. of serum of a sensitive animal injected into a "fresh" animal rendered it in turn hypersensitive after the usual incubation period of fifteen days. They also found that the blood of one sensitized and subsequently immunized animal transferred to another hypersensitive animal contains no demonstrable toxic substance. If, on the other hand, the blood of the refractory animal is transferred to a fresh animal it sensitizes it after the usual incubation period. Otto has recently published experiments showing that a fresh animal may be rendered hypersensitive within twenty-four hours by the injection of the blood serum of a hypersensitive guinea-pig. Bearing on these points I submit the following tabulated results of experiments on the passive transfer of the hypersensitive condition from animal to animal.

I have not thought it necessary to detail the history of the animals from which the sensitizing blood was drawn. They were all guinea-pigs that had been through the treatment with a toxin-antitoxin mixture some weeks previously. In order to eliminate a possible individual variation in the blood of different animals a mixture of bloods was usually employed. As it was unknown whether the intermediate substance was a labile body or not, and whether it was free in the serum or might not perhaps be bound to corpuscles, many of my attempts to transfer were made with freshly defibrinated blood including the corpuscles.

The results show definitely that there is in the defibrinated blood and in the blood serum of guinea-pigs hypersensitive to horse serum a substance which, when injected into normal guinea-pigs, renders them also hypersensitive to horse serum after a lapse of twenty-four hours.

The further study of the characteristics of this substance must be left to the future. One experiment shows that it is not destroyed by heating the serum to 60° C. for half an hour. Otto has found that it has no power to divert complement when combined with horse serum.

Gay and Southard's observation that a smaller quantity of sensitive or refractory serum can sensitize after the incubation period, I am able to confirm. I agree with them in the belief that this is a manifestation of a retained element of horse serum. But I think that this acts as an active sensitizer and is entirely distinct from the anti-body which takes part in the intoxication.

V. IMMUNITY OR ANTIANAPHYLAXIS.

It was very soon found that if a hypersensitive animal were injected with a large dose of serum but survived the reaction, a second large dose within a few days produced less reaction or none at all. Otto, who first recorded this observation, dismissed the matter in a sentence by assuming that the toxic substance is a haptin in the Ehrlich sense. It has since been found that the reaction is very different in its time relations, at least, from other immunity reactions. Twenty-four hours, or perhaps less, is all the time required to bring this immunity to its full protective force, as has been pointed out by Besredka and Steinhardt. The first of the following experiments is illustrative. Comparison of the first with the second experiment shows, too, that it is the quantity of serum injected and the point of injection that are important, rather than the fact that the animal has survived the reaction.

G. P. 4764. Sensitized Jan. 29, 1907.

$\frac{\text{Diph. Toxin .21 c.c.}}{\text{Serum 1/175 c.c.}}$ Small ulcer; paralysis.

March 28, 1907, 11 a. m. Serum of normal horse No. 93; 0.5 c.c. injected subcutaneously.

6 p. m. Quite sick.

March 29, 1907, 10 a. m. Well.

5.0 c.c. normal serum of horse No. 93; injected subcutaneously. No symptoms.

G. P. 5014. June 8, 1907. Sensitized.

$\frac{\text{Diph. Toxin .215 c.c.}}{\text{Serum 1/300 c.c.}}$ No lesion.

June 28, 1907: Normal serum of horse No. 106.

1/200 c.c. serum + 199/200 c.c. salt sol. by intracardiac injection. Severe symptoms; convulsions.

June 29, 0.1 c.c. ser. + 0.9 c.c. salt sol. by intracardiac injection. Dead after 3 minutes.

Besredka and Steinhardt have shown that it is possible to immunize in twenty-four hours against the more delicate intracranial injections and I have been able to extend this to the intracardiac injection.

The condition which makes it possible to reduce so rapidly the hypersensitiveness in the animal I believe to be a local hypersensitiveness of the subcutaneous tissues, which tends to hold the active substance of the serum at the point of injection, and so greatly to lessen its absorption rate. If the serum be so gradually introduced that this local reaction is effective, the anti-body on which the hypersensitive reaction depends may be entirely neutralized without killing the animal, or even rendering it appreciably sick, even though the test serum be introduced into the circulation. The following experiment demonstrates these points.

Three hypersensitive guinea-pigs[2] were treated as follows:

Sept. 19:	8 p. m.	0.5 c.c. Normal horse serum, subcutaneously.	No symptoms.
Sept. 20:	8 a. m.	2.0 c.c. Normal horse serum, subcutaneously.	No symptoms.
Sept. 20:	12 m.	5.0 c.c. Normal horse serum, subcutaneously.	No symptoms.
Sept. 20:	8 p. m.	5.0 c.c. Normal horse serum, intraperitoneally.	No symptoms.

September 21, 10 a. m. One of the animals received 1.5 c.c. normal horse serum by intracardiac injection; no symptoms. The other two animals were now bled, the blood was defibrinated and 15 c.c. was injected into the peritoneal cavity by a fresh normal guinea-pig weighing 240 grm. September 22, 4 p. m. After an interval of 30 hours this last animal received 1.75 c.c. normal horse serum by intracardiac injection; no symptoms.

This experiment is controlled by those on guinea-pigs Nos. 4975, 6010, 5079, 6017, 6058, 6059, of Table III. It shows conclusively that the substance on which the passive transfer of the hypersensitive reaction depends is removed from the circulation of the hypersensitive animal by the gradual introduction of large amounts of horse serum. The experiment could equally well have been considered in the section on the passive transfer of the hypersensitive state as showing that the anti-body there demonstrated was really a vital factor in the acute reaction.

As above stated, I believe that it is a local hypersensitiveness in the subcutaneous and peritoneal tissues which makes possible this

[2] These animals were the same which were used to obtain the serum to sensitize guinea-pigs Nos. 6058 and 6059. See Table III.

rapid neutralization of the anti-body without general symptoms. Under certain conditions which are not as yet fully determined this local hypersensitiveness may be greatly exaggerated. The animal is then fully protected against the acute intoxication, but its life is later sacrificed to the severe reaction in the subcutaneous tissues and abdominal organs. This type of reaction, well known in the rabbit, has not been observed heretofore in the guinea-pig, and I will, therefore, describe the cases which I have encountered in some detail.

In a number of instances I have departed for one or another reason from the usual preliminary or sensitizing treatment. In four cases I repeated, after a number of weeks, the original toxin-antitoxin mixture as nearly as might be. In twelve cases I fed serum by mouth to hypersensitive animals. In eight cases, reported above, repeated small doses of serum were given over a period of several days as a sensitizing treatment. It is not possible to discuss at present all of these experiments from the point of view from which they were undertaken. But the animals had one interesting feature in common when subsequently tested with five or six cubic centimeters of normal serum by the subcutaneous method. Several of the animals of each group died acutely with the usual symptoms. Three of the animals that received serum by mouth gave no reaction whatever. All of the remaining animals showed one or another phase of the following reaction. Acute symptoms following the injection were present or absent, but in all cases in which they were present the animal practically recovered from them in eight hours. At this time also the injected fluid was about absorbed, so that the subcutaneous tissues showed at most but a trace of thickening. From now on the animals became worse again. They became drowsy and had a staring coat with very watery eyes. Locally by the end of twenty-four hours after injection, there was a well-marked œdema, in some instances a very large one. In the milder cases the œdema was reabsorbed and the animal recovered in four or six days. In the severe cases the œdema became very large. Two animals died on the third day with a spreading œdema covering the whole abdominal and thoracic region. In the animals that lived beyond the third day, the œdema gradually became

harder, the overlying skin underwent a dry blackening necrosis, and finally the affected area sloughed out, leaving a bare ulcer varying with the severity of the case from one half to three inches in diameter. These ulcers were very slow to heal, the smaller ones taking a month, the larger ones three months from the time of injection to complete repair. These animals, as they have died, have been studied in gross and microscopically, and others have been chloroformed at one or another stage to complete the series. The results can be briefly stated.

The local lesion is at first an œdema of the subcutaneous tissues and abdominal muscles without cellular invasion. This is associated after several days with pronounced degeneration and necrotic changes in muscular tissues, connective tissues and skin. A dry superficial eschar overlying the œdematous subcutaneous tissues is formed. Through the breaks in the escharotic skin, bacteria gain an entrance. This infection developing on the fifth or sixth day calls forth a leucocytic reaction. The necrotic tissue is thrown off and leaves a bare ulcer, which, as has been said, heals very slowly. Internally one finds remains of the acute changes in the lungs and gastro-intestinal tract which have been so fully described by Gay and Southard. In harmony with the fact that the acute symptoms in these cases are slight, the lung lesions are always very small. The gastric and intestinal lesions are, on the other hand, very extensive. In two instances an irregular hemorrhagic ulceration occupying fully two thirds of the stomach wall was found. The lesion of the stomach has not been found in its stages of repair. In severe cases on the third day it is interesting to note that bacterial invasion has begun, and that a leucocytic reaction is only found at points where the bacteria have penetrated well within the necrotic gastric mucosa.

In the acute cases the lymphatic apparatus never displays definite pathological alteration, but in these cases of late reaction the spleen and mesenteric lymph nodes show interesting changes. The spleen frequently shows considerable areas of hemorrhagic necrosis. In one instance three-fourths of the organ was involved. Microscopically, the affected areas show extensive hemorrhage. The extravascular corpuscles and those in neighboring vessels are clumped,

fused, and often laked. The connective tissues and leucocytes in the affected areas are in various stages of degeneration. Where the necrotic areas border healthy tissue there is well-marked invasion of the hemorrhagic area by phagocytic endothelial cells. The healthy spleen tissue shows endothelial cell proliferation.

The mesenteric lymph nodes on the second and third day after injection show moderate œdema and congestion. The germinal areas show no alteration. The peripheral sinus and those at the hilum are dilated with serous fluid containing desquamated endothelial cells and red blood corpuscles in moderate number. In one instance a few threads of fibrin were found. The endothelial cells free in the sinuses are in stages of degeneration by lysis, and the red blood cells are agglutinated in clumps about them. The endothelial cells lining the sinuses are swollen, raised from the connective tissue cells backing them or in places are wanting altogether. In some instances the red blood corpuscles are clumped about cells that are still attached to the sinus wall. The bone marrow has been studied, but similar changes have not been found. These lesions of the lymphatic apparatus will receive more extended discussion in another paper.

Finally, I have paid particular attention to the condition of the blood in the vessels in all of the animals which I have been able to autopsy at the time of their death. The blood has frequently been drawn, suspended in salt solution and examined microscopically for evidence of agglutinative clumps. The vessels have been traced deep into the lungs in search for thrombi, and the sections have been carefully examined for the same. My conclusions are that fibrinous thrombi are never found and that such clumping of red blood cells as occurs is not enough to account for the lesions with which it is rather irregularly associated. The clumping as well as the hemorrhage are probably secondary to endothelial cell degeneration. The relation of this late reaction to the acute reaction will be further discussed with the theoretical considerations in a subsequent paragraph.

VI. GENERAL DISCUSSION OF PROBLEM.

I wish now to restate briefly the main facts in the case and to offer an explanation for them in so far as it seems possible to do so with our present knowledge.

The normal guinea-pig is not injured by the injection of normal or antitoxic horse serum into its body in any amount that the mechanical conditions at the site of inoculation will permit of. If, however, a normal guinea-pig be first treated with a small amount of normal horse serum and after a time be injected with a large quantity, it will become very sick or die. If it does not die it will recover rapidly. Within certain limits of quantity and time the larger the first or sensitizing treatment, the less injurious is the second test, or intoxicating injection. If a third dose, large or small, of horse serum be given twenty-four hours or more later, it is less apt to injure than the second dose, and this applies to all subsequent subcutaneous injections of horse serum. Female guinea-pigs which have been treated with horse serum one or more times whether themselves injured or not, transmit a hypersensitiveness to their offspring. The blood or blood serum of a hypersensitive animal if transferred by injection into a normal guinea-pig in a suitable dose, renders this animal also hypersensitive. This takes place within twenty-four hours if the dose be large enough, but with a dose that is ineffective at this time the sensitization can be accomplished after the same incubation period as that required for the injection of a small dose of horse serum to become effectual. Under various conditions, which all involve repeated treatments with small doses of serum before any large dose is given, the animals may be found hypersensitive in the usual way, or they may develop a more local reaction and die or recover after a longer time. The minor facts in the case will be referred to in the course of the following discussion.

The only attempt to explain comprehensively the mechanism of this particular reaction by one who has experimented with it is that of Gay and Southard. They take the view that the sensitizing substance in the horse serum is distinct from the toxic substance, but a critical study of their experiments does not reveal an adequate basis for this assumption, which in the absence of demonstrated facts in its support is unnecessary. They suggest that chemical analysis may support their view, but at present there seems neither more nor less reason for supposing that the toxic and sensitizing elements in horse serum are distinct substances than for assuming that diph-

theria toxin is really a mixture of a toxic substance responsible for its injurious effects and another substance which stimulates the production of anti-bodies. They have admittedly demonstrated by an experiment that is easily repeated, that the sensitizing principle of the horse serum remains for a very long time in the body of the animal into which it is injected, and that it can be transferred to the body of a second animal mechanically by the transfer of blood serum. That this substance acts as a sensitizer by irritating certain cells and increasing their affinity for the toxic substance (to use their terms) can be granted. But that is less definite than the statement that by injury it stimulates these cells to the production of an excess of receptors which are in part, at least, cast off and appear in the circulating blood as an anti-body. It is more probable that the offspring of hypersensitive female animals are hypersensitive because of the presence of this anti-body than because of the presence of the irritating serum constituent, as these workers suppose. On their supposition, the young should remain sensitive throughout their lifetime and transmit sensitiveness in favorable cases to the grandchildren. If experiment has not rigidly excluded these possibilities, it has at least rendered them very improbable.

There is at present, as I understand it, but one serious biological objection to the view that the toxic substance in small quantity is the sensitizing substance. The ultimate function of the anti-body produced must be assumed to be the elimination of the toxic substance. Why, then, since it is produced in such excess, does it not do this completely, very soon, and allow the hypersensitive condition to disappear? By supposing that the toxic substance was closely united to body cells at some stage of its elimination after uniting with the anti-body, either by extreme solubility in or close chemical affinity for certain of their constituents, one could understand the retention of a residual quantity sufficient to prolong the sensitive condition. But while there is no satisfying evidence against the unity of the substance in the serum, the work of Vaughan and Wheeler on egg-white has rendered it almost certain by analogy that the substance of the serum is chemically decomposed in the body of the animal so as to leave a non-toxic residue difficult of elimination, which keeps up the sensitization.

As I would state it provisionally, there is in horse serum a substance which is actually a mild and potentially a severe toxin for the somatic cells of the guinea-pig. It is actually a mild toxin because receptors to link the toxic substance to the cells are almost wanting. By the introduction of a minimal amount of this substance, the few receptors normally present are exhausted, and subsequently regenerated in great excess. They can now be transferred passively either from mother to offspring or mechanically with the blood serum. When the intoxicating dose of serum is injected a relatively large amount of the toxin can now be suddenly brought in contact with the susceptible cells and the acute reaction is developed. It must be remembered that this is a case in which the substance involved is not toxic for cells essential to the life of the animal when gradually administered, but only becomes so when it is suddenly introduced in excess.

There has been so far no opportunity to study the characteristics of this anti-body. It seems to be necessary to introduce the sensitive guinea-pig serum some time before injecting the horse serum if the reaction is to run at a rate that will injure the animal. This would indicate that the anti-body must be united to some constituent of the body cells before the horse serum is introduced if the animal is to be effectively sensitized. I have been able to show by one experiment that this antibody is not a very labile substance, but the details must be further developed.

It will be most instructive, perhaps, to consider for a time the hypersensitive animal as though it were a normal animal very susceptible to a particular toxin. If this animal is subjected to treatment with a considerable but not fatal dose of toxin it is subsequently found to have lost its susceptibility. The first thought, reasoning from analogy, is that it has acquired an immunity in the special sense of the term. The fact that this immunity is not transferred by the mother to her offspring and that the blood of such an animal has no protective value for an animal not immune, is not an argument against the animal itself being protected by anti-bodies against the toxin or some combination or reaction product of it. The cases in which such anti-bodies can be demonstrated by passive transfer while numerous do not even cover the whole field of immunity against bacteria.

But in this instance the facts in regard to the immunity, if it is well to apply the term to the condition, are adequately explained without assuming that such anti-bodies are formed.

Another well-known although not perfectly understood reaction by which a susceptible animal can be protected against a toxic or infectious agent is by an increase in the affinity of cells not essential to the life of the organism for the toxin. This may be manifested by the leucocytes in conjunction with a special class of anti-bodies, or by the subcutaneous tissues. The mechanism of the reaction of the latter tissues is not thoroughly worked out. In this instance I believe it probable that the late reaction with necrosis before described is an example of this form of protection. By modifying the sensitizing treatment the affinity of the subcutaneous tissues is probably raised to a point where they absorb and hold so much of the toxin that very little can reach the circulation and be carried to cells more vital to the life of the organism. If they are sufficiently hypersensitive they do this to their own destruction. This case is perfectly explained in this way if it be assumed that the effective receptors are retained within the cell, and that those in the circulation represent but an unessential fraction of the total.

Nicolle experimented further with the necrosis which Arthus first produced in the subcutaneous tissues of rabbits by repeated injections of horse serum. He found that following the early treatments there was developed an anti-body which when transferred passively with the blood serum to a fresh animal caused it to react to a first subcutaneous serum injection with necrosis. He has not tried the effect of intravenous injections on either the actively or passively sensitized rabbits, but it seems probable that the reaction is essentially the same as that in the guinea-pig, with the difference that the rabbit's subcutis is easier to sensitize to a point where it will protect the animal's life at its own expense. That the "Phenomenon of Arthus" is in its essential features identical with the "Theobald Smith Phenomenon" is the recently expressed opinion of Otto also. Neither is this the only intoxication in which the rabbit's subcutis exhibits a greater binding power for the poison than does that of the guinea-pig. Morgenroth(12) showed that this was the case for diphtheria toxin as well.

In the case of this particular reaction there is a third possible explanation for the failure of the animals to react for a period. Depending for its effect in large part on a rapid reaction rate, it can be understood that if the serum is introduced very slowly all of these receptors can be satisfied without injury to the animal. The hypersensitive animal then becomes neither immune nor refractory, but is for the time being normal, or rather is a normal animal with a recent large dose of serum. This result for the animal is the reverse of that which is brought about in the course of certain procedures in the immunization against bacteria. For a short time after a given treatment of a fresh or partially immunized animal, the animal may be more than normally susceptible to infection. This is supposed to be due to an exhaustion of the protecting receptors which are not yet sufficient in quantity to protect effectively against the dose administered and still leave a surplus. It is the same in this instance, except that as the anti-body or receptor is a detrimental rather than a protective agent, its removal is salutary. That the mere exhaustion of the abnormal receptors explains the immunity, or, as they term it, the antianaphylaxis, is the view of Besredka and Steinhardt, reasoning from the fact that the animals after a time become sensitive again. For these workers, however, the whole reaction takes place in the nervous system, while my impression is that the nerve cells take little part in the reaction except as they may be subjected to actual injury by the rapid exhibition of the toxic substance in the hypersensitive animal.

It is, perhaps, needless to emphasize the point that the explanation above offered is only intended to cover the facts in this particular reaction in so far as they have been experimentally developed. Other hypersensitive reactions seem to be more complicated, and more complex explanations have been offered for them. It would be unwise to impose such theories on the phenomenon here discussed in advance of the demonstration of facts requiring them.

A few words should be said in closing about the pathological anatomy of the serum intoxication. The work of Gay and Southard was instructive in showing the rapidity with which certain definite and important pathological alterations in tissue cells may be developed. My own studies also have shown conditions which are

interesting from the point of view of the specialist in pathological anatomy. But the significance of these changes in a general consideration of the subject remains doubtful. While Gay and Southard were able to show definite cellular lesions four minutes after injection, and while hemorrhages are not uncommon at that time, yet it is true that in the cases in which death occurs most quickly, lesions are much less frequent and widespread than in those in which it is delayed for several minutes or hours. It would probably be possible to kill animals in this way without demonstrable lesion. In most of the early cases, at least, I think the cause of death is rather to be referred to the disordered function of a single organ, or broken coordination between several organs than to anatomical lesion of any one organ or cell complex. Only pharmacological methods could show whether the action is on the nervous system. the heart, or the lungs directly, or on the heart and lungs through the nervous system. Anatomical studies show that whatever may be the immediate cause of death the toxic substance in its absorption, transmission and elimination injures to a greater or less degree cells of many types.

VII. SUMMARY.

Following the divisions before used, the results presented in the preceding pages may be briefly stated.

I. The particular method of sensitization and the place where the test injection is made have an important bearing on the results obtained by various workers. Comparing the results obtained by the various methods, we may conclude that the incubation period of the hypersensitive reaction is not sharply limited, but that there is a progressive increase in sensitiveness from the sixth day, and presumably before that, extending over a period of several weeks. It seems very probable that the degree of hypersensitiveness attained where the sensitizing dose consists of a mixture of diphtheria toxin and serum is greater than when a single dose of the same small quantity of serum is given alone.

II. Our early experiments, the first in this field, are in thorough agreement with those first reported by Otto, and shortly after him by Rosenau and Anderson.

III. This hypersensitive reaction is transmissible from mother to

offspring. The transmission is probably not equally effective in all cases, and individual young guinea-pigs probably vary greatly in the rate with which they lose their ability to react. As a result, not all of the young of a hypersensitive mother react to a subcutaneous dose of five cubic centimeters of serum given when they are four or five weeks old. The reaction in the young animals differs quite markedly from that in those actively sensitized. These differences are such as to indicate that in the mother there is a considerable localization of the reaction in tissues and organs whose destruction does not cause sudden death. This local reaction is a protective factor and is not transmitted to the same degree as the factors involved in the fatal acute reaction.

IV. The hypersensitive reaction to horse serum depends on the development of a special anti-body during the incubation period, which anti-body may be passively transferred to a fresh animal. If the dose of hypersensitive serum be sufficient, and the intoxicating injection be given directly into the circulation, this passive hypersensitiveness may be enough so that the animal will die when tested. There is also in the serum of hypersensitive guinea-pigs an uneliminated horse serum element or "rest," which is distinct from this antibody, and probably without influence on the course of the acute reaction.

V. The anti-body on which the hypersensitive reaction depends may be entirely neutralized by horse serum without causing symptoms. The gradual introduction of increasing doses over a total period of twenty-four hours suffices for this. The animal is then, properly speaking, neither immune nor refractory, but is essentially in the condition of a normal animal which has recently had a large dose of horse serum. This rapid neutralization is made possible by the great binding power which the subcutaneous and other relatively unimportant tissues have for the toxic element of the serum. The so-called "Phenomenon of Arthus" is probably the same reaction for the rabbit that we have here dealt with in the guinea-pig. The fact that the manifestation is more prominently a local one depends on racial differences. I have encountered cases in the guinea-pig in which the conditions in the rabbit are closely simulated.

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ON THE INHIBITORY INFLUENCE OF EOSIN UPON SPORULATION.[1]

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Sporulation requires optimum temperature and suitable nutrient media. It is greatly influenced by various physical and chemical agents. Thus, in the case of Bacillus anthracis, the most studied in this respect of all spore-bearing organisms, no sporulation takes place at temperatures above 42° C.[2] or below 14° C.[3] Weil[4] places the lowest temperature at which sporulation takes place at 7° C. The presence of oxygen seems to be essential to the formation of anthrax spores.[5] Persistence of conditions unfavorable to sporulation through many successive generations of the organism gives rise to asporogenous strains in which virulence is often found greatly reduced or even totally absent.

Phisalix[6] succeeded in obtaining an asporogenous stain of B. anthracis by cultivating it at 42° C. for twelve successive generations. Roux[7] induced a similar biological alteration by means of a medium containing certain chemicals. Potassium bichromate in the ratio of 1 to 2000, or phenol in from 2 to 6 to 10,000 added to ordinary bouillon stops sporulation completely. Behring[8] found that various acids, alkalies, salts and certain antiseptics when used in suitable concentrations prevent sporulation. The antisporulative property of certain dyes was also described by Behring,[9] who states that safranin in 1 to 30,000, and methylene violet, cyanid, and malachite green in 1 to 200,000 to 1 to 600,000 exert a powerful restraining influence upon the growth and sporulation of B. anthracis. After

[1] Received for publication October 1, 1907.

[2] Phisalix, *Bull. méd.*, 1892, vi, 533.

[3] Kitasato, *Zeit. f. Hygiene*, 1890, viii, 198.

[4] Weil, *Zeit. f. Hygiene*, 1901, xxxvi, 451.

[5] Schreiber, *Cent. f. Bakt.*, 1896, xx, 353. Weil, *Arch. f. Hygiene*, 1901, xxxix, 205. Klett, *Zeit. f. Hygiene*, 1900, xxx, 420. Jacobitz, *Cent. f. Bakt.*, 1901, xxx, 232. Slupski, *Cent. f. Bakt.*, 1901, xxx, 396.

[6] Phisalix, *Bull. méd.*, 1892, vi, 533.

[7] Roux, *Annales de l'Inst. Pasteur*, 1890, iv, 25.

[8] Behring, *Zeit. f. Hygiene*, 1889, vi, 117.

[9] *Idem*, 1889, vii, 171.

two months' successive cultivation in the colored agar media, no permanent loss of the sporulating property resulted.

Schreiber[10] states that potassium phosphate of a concentration above 3 per cent. prevents sporulation of B. anthracis, B. subtilis and B. tumescens. Behring,[11] Bormans,[12] and Lecleff[13] found that B. anthracis does not form spores in blood serum, while Brieger, Kitasato and Wasserman[14] recorded a few instances in which B. tetani failed to sporulate in an aqueous extract of thymus gland.

While abundant work has been done with various spore-bearing aerobes, especially with B. anthracis, a similar study with anaerobic organisms has been so far neglected. In a recent study on the antitetanic property of certain dyes, Flexner and Noguchi[15] called attention to the fact that eosin in an adequate strength prevents the sporulation of B. tetani, the experimental details of which are given in a later paper by Noguchi.[16]

In the present communication I wish to present some of the results of experiments on the restraining influence of eosin upon the sporulation of various microbes. The varieties of bacteria subjected to experiment belonged to the aerobic and to the anaerobic organisms. Of the first, B. anthracis, B. megatherium, B. cereus, B. mesentericus, B. subtilis, B. ruminatus, and B. anthracoides, and of the second, B. tetani, B. anthracis symptomaticus, B. botulismus, B. œdema maligni, B. enteritidis sporogenes and B. putrificus were studied.

Eosin "Gelb" having been mixed with agar or bouillon in varying strengths, the inoculations of bacteria were made as usual. Stab and slant solid cultures were made. For the anaerobic bacteria, tissue-bouillon and a deep layer of glucose agar were employed. The agar tubes were incubated in an atmosphere of hydrogen. The results are tabulated.

Table I. indicates that the inhibitory action of eosin is most intense upon B. cereus and B. mesentericus, and least upon B. anthracoides, while B. subtilis, ruminatus, anthracis and megatherium occupy intermediary positions. All growth became uncertain when the concentration of eosin reached one per cent.; below this

[10] Schreiber, *Cent. f. Bakt.*, 1896, xx, 353.
[11] Behring, *Zeit. f. Hygiene*, 1889, vi, 117.
[12] Bormans, cited in *Baumgarten's Jahresberichte*, 1895, xi, 138.
[13] Lecleff, *La Cellule*, 1894, x, 349.
[14] Brieger, Kitasato and Wassermann, *Zeit. f. Hygiene*, 1892, xii, 137.
[15] Flexner and Noguchi, *Jour. of Exper. Med.*, 1906, viii, 1.
[16] Noguchi, *Jour. of Exper. Med.*, 1907, ix, 281, 291.

TABLE I.—*Examination after 10 Days.*

Slant Agar with Eosin "Gelb."	B. cereus.	B. mesentericus.	B. subtilis.	B. ruminatus.	B. anthracoides.	B. anthracis.	B. megatherium.
Control (no eosin).	+ all	+ all	+ all	+ all	+ all	+ all	+ all
0.001 per cent.	+ few	+ few	+ many	+	+	+	+
0.01 "	—	—	+ few	+	+	+	+
0.05 "	—	—	—	+?	+	+?	+?
0.1 "	—	—	—	—	+	—	—
0.5 "	—	—	—	—	—	—	—
1 "	—	—	—	—	—	—	—
2 "	—	—	—	—	—	—	—

+ = spore formation.
— = no spore formation.
+? = sporulation doubtful.

strength, multiplication of the bacteria still takes place. These organisms, when cultivated in bouillon containing varying amounts of eosin "Gelb," appear to be even more sensitive to the action of the dye than when grown on a slant agar surface. But when the cultures are allowed to stand for many weeks, sporulation takes place in a medium containing as high concentration of the dye as 0.1 per cent. Concentrations above 0.3 per cent. prevent sporulation, to which effect is added restraint of growth and formation of chains of bacteria through imperfect multiplication. At times, marked degrees of involution occur. In no instances was growth discovered in bouillon containing two per cent. of the eosin.

In deep stab cultures the results were practically identical with those given in the table. Transplantation of the asporogenous bacilli into eosin-free media was associated with immediate return of the spore-bearing power. Many weeks' contact of the sporeless vegetative bacilli with the eosin exerted no enduring effect on the sporogenous property.

The influence of eosin "Gelb" on the sporulation of the anaerobic species has been shown in Table III. Recapitulated, they show that

TABLE II.

Bouillon Culture with Eosin "Gelb."	B. cereus.	B. mesentericus.	B subtilis.	B. ruminatus.	B. anthracoides.	B. anthracis.	B. megatherium.
Control (no eosin)	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 25 d. +	3 d. + 25 d. +
0.001 per cent.	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 25 d. +	3 d. + 25 d. +
0.003 per cent.	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. — 25 d. +	3 d. — 25 d. +
0.01 per cent.	3 d. — 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. — 25 d. + few	3 d. — 35 d. +
0.03 per cent.	3 d. — 38 d. +	3 d. + 38 d +	3 d. — 38 d. +	3 d. + 38 d. +	3 d. + 38 d. +	3 d. — 25 d. + few	3 d. — 25 d. +
0.1 per cent.	3 d. — 38 d. +	3 d. — 38 d. +	3 d. — 38 d. + } No surf. membr.	3 d. — 38 d. +	3 d. — 38 d. +	3 d. — 25 d. + ?	3 d. — 25 d. — } No surf. membr.
0.3 per cent.	3 d. — 38 d. —	3 d. — 38 d. —	3 d. — 38 d. + } No surf. membr.	3 d. — 38 d. —	3 d. — 38 d. —	3 d. — 25 d. — ?	3 d. — 25 d. — } No surf. membr.
1 per cent.	No growth	3 d. — 38 d. —	3 d. — 38 d. —	3 d. — 38 d. —	3 d. — 38 d. —	3 d. — 25 d. —	No growth
	No growth	No growth.	No growth	No growth	No growth	No growth	No growth

d = day or days. + = spore formation. — = no spore formation.

TABLE III.—*Observations made after 29 Days on Certain Anaerobes Cultivated Aerobically in the Presence of Tissue.*

Bouillon Containing a Small Piece of Rabbit's Fresh Liver.	B. tetani.	B. anthracis symptomaticus.	B. botulismus.	B. oedema maligni.	B. enteritidis sporogenes.	B. putrificus.
Percentage of eosin "Gelb" in the bouillon						
Control (no eosin)	+	+	+	+	+	+
0.001 per cent.	+	+	+	+	+	+
0.003 per cent.	+	+	+	+	+	+
0.01 per cent.	—	+ ?	+	— ?	—	—
0.03 per cent.	—	—	—	--	—	—
0.1 per cent.	—	—	—	—	—	—
0.3 per cent.	—	—	—	—	—	—
1 per cent.	No growth	No growth	No growth	No growth	No growth	No growth

+ = spore formation.
— = no spore formation.
? = spore formation doubtful.

the eosin in the strength of one per cent. completely inhibits all multiplication of the bacteria. The results are only very slightly different in the case of the cultivation of the anaerobic species freely in the air, in the presence of tissue (Table III.) and in an atmosphere in hydrogen in deep glucose agar. The phenomena of restraint are less pronounced in the latter media.

SUMMARY.

Sporulation of B. anthracis, B. subtilis, B. cereus, B. ruminatus, B. mesentericus, B. anthracoides and B. megatherium does not take place in an agar medium containing eosin "Gelb" in a concentration exceeding 0.5 per cent. In a concentration of 0.1 per cent. most of these bacteria fail to produce spores. The greatest sensitiveness is shown by B. cereus and B. mesentericus. In a bouillon medium sporulation is likewise inhibited by eosin, but after a longer time—seven weeks or more—sporulation still occurs where the concentration of the dye equals one-tenth per cent.

Sporulation of B. tetani, B. anthracis symptomaticus, B. botu-

lismus, B. œdema maligni, B. enteritidis sporogenes, and B. putrificus does not take place in a medium containing eosin "Gelb" in concentrations exceeding 0.03 per cent. With these organisms, no difference was noted in the final effect, depending on the medium employed. No permanent loss of power to produce spores ensues with the bacteria tested even after long sojourn in the eosinized media.

It may be stated that on the whole the inhibitory action of eosin was more pronounced upon the anaerobic than upon the aerobic species of bacteria employed in these experiments.

THE TRANSPLANTATION OF HUMAN CARCINOMATOUS MATERIAL INTO LOWER ANIMALS.*

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Although many attempts have been made to inoculate successfully the lower animals with portions of tumors from human beings they have in the great majority of cases been without results. In some instances the observers have claimed that they have been successful but their statements are, as a rule, not convincing.

Carl Lewin[1] discusses this question of transplantation at length and reviews the reports of those who have conducted such experiments. The following is a synopsis of the instances that he considers, separating them into two groups, according as the results of the transplantation were negative or apparently successful.

The first to be given are the negative instances.

Doutrelepont[2] experimented with the transplantation of human tissue into the lower animals with negative results.

Billroth[3] attempted to transfer human carcinoma and round cell sarcoma to dogs. No growth was however, obtained either by implantation or by injection into the jugular vein. Lebert and Wyss[4] injected a sarcoma emulsion into rabbits without any growth taking place. Fischl[5] injected rats intraperitoneally, intravenously and subcutaneously with a small cell sarcoma of the upper arm and with a melano-sarcoma of the lymph nodes. The tumors were absorbed without leaving the slightest trace. Duplay and Cazin[6] and Pawlowsky[7] transplanted sarcomatous tissue with no resulting growth. Roux and Metschnikoff[8] inoculated portions of a melano-sarcoma into the anterior chamber of the eye, and also beneath the skin of a young chimpanzee, with negative results.

* Received for publication October 14, 1907.

[1] Lewin, *Zeit. f. Krebsforsch,* 1906, iv, 55.

[2] Doutrelepont, *Virchow's Archiv,* 1869, xlv, 501.

[3] Billroth, *Wien. med. Woch.,* 1867, xvii, 437, 453.

[4] Lebert and Wyss, *Virchow's Archiv,* 1867, xl, 142, 532.

[5] Fischl, *Fortscritte der Medizin,* 1892, x, 159.

[6] Duplay and Cazin, *Semaine médicale,* 1892, xii, 61.

[7] Pawlowsky, *Virchow's Archiv,* 1893, cxxxiii, 464.

[8] Roux and Metschnikoff, *Bull. de l'Academie de med.,* 1903, s. 3, l, 101.

Many positive reports are given; they include the following.

Follin and Lebert[9] injected from sixty to seventy grammes of a mixture of mammary carcinoma and water into the jugular vein of a dog. At the end of fourteen days nodules about the size of a bean were found in the wall of the heart and tumors the size of a pin's head were found in the liver. No definite microscopic report is given.

O. Weber[10] injected portions of a carcinoma of the superior maxilla into the vena cruralis of a dog and also inoculated the same animal subcutaneously. On the sixth day granulations appeared at the site of the inoculation and continued growing until a mass the size of a man's fist was formed. As the granulation mass began to undergo necrosis the animal died. Similar granulation tissue was formed in a cat. Gouyon[11] obtained positive results from inoculation into dogs and guinea-pigs. Klencke[12] claims to have successfully inoculated horses and dogs with melanotic tumor cells.

Langenbeck[13] injected, on June 8, intravenously into a dog, cancer masses suspended in blood serum. On August 10 he killed the animal and found on the anterior surfaces of the upper lobes of both lungs two or three minute flat tumors that microscopically showed the structure of carcinoma. In the middle lobe of the left lung there was a hard mass about the size of a bean that also showed carcinomatous formation. Microscopically the growth consisted of a net-work of fine fibers between which were masses of closely packed cells. Lanz[14] injected a few drops of an emulsion of melano-sarcoma into the spleen of a dog. The animal became obviously cachectic and died at the end of one and a half months. A black mass at the site of the injection was found at autopsy. The subcutaneous tissue was slate-gray in color, the entire peritoneum dark, and the spleen deep black and soft. Pigment deposits were found in the liver, intestines, serosa, kidneys, lungs and epicardium. The pigment was generally of a deep black color, but in places it was greenish. It existed mostly free in the tissues but partially within the cells. Further transplantations were negative.

Lanz[15] also inoculated several rabbits with a peculiar tumor of the membranes of the central nervous system. After four months one animal showed a rapidly growing tumor in the left eye. After death the following conditions were observed: In the fatty tissue of the orbit there was found a stratified growth that had caused some necrosis of the neighboring bone. In the lungs and the kidneys and over the peritoneum, especially, were numerous soft tumors. Microscopically there was found "a parasitic infection of nearly all the endothelial cells of the peritoneum." The orbital tumor showed microscopically the structure of a sarcoma of the iris or choroid. The author believed that he had found in the parasite the cause of certain sarcomatous neoplasms.

[9] Follin and Lebert, *Virchow's Archiv,* 1867, xl, 538.

[10] Weber, Chirurgische Erfahrungen und Untersuchungen, Berlin, 1859, p. 289.

[11] Goujon, *Jahresbericht u. d. Leistungen d. gesamt. Medizin,* 1867, ii, pt. 1, 289.

[12] Klencke, *Archiv f. d. gesamt. Medizin,* 1843, iv, 484.

[13] Langenbeck, *Schmidt's Jahrbücher,* 1840, xxv, 99.

[14] Lanz, *Festscrift für Kocher,* 1891, p. 299.

[15] Lanz, *loc. cit.*

Jürgens[16] removed portions of *Sarcoma melanoticum carcinomatoides* thirty-six hours after death and implanted them into rabbits. In one animal he found at the end of eight days small tumors in the omentum and the mesentery. These had formed around the implanted tissue and consisted of large round cells and polymorphic cells. A second rabbit at the end of three weeks showed in the omentum two tumors about the size of a cherry stone. A third animal had small black tumors in the mesentery; and on the epicardium over the right heart some black soft structures about the size of a hemp seed.

Jürgens further reports the successful implantation of human sarcomatous tissue into rabbits. After intraperitoneal transplantation of a metastatic sarcoma of the brain there developed in the right eye of the rabbit a tumor the size of a hazel-nut of the same structure as the original growth. A second rabbit that had been inoculated in the eye with a melano-sarcoma, showed in the course of fourteen days, a tumor the size of a pea. He also succeeded in obtaining tumors in the lungs and intestines of a rabbit by inoculating it, twenty-four hours after its removal at autopsy, with a myxo-sarcoma of the ovary.

Mayet[17] reports that by injecting animals with the juice of malignant tumors he was able to obtain tumor growths. Von Bambeke[18] saw a tumor form in a rat five weeks after the transplantation of sarcomatous tissue. Reale[19] implanted subcutaneously a piece of a *Sarcoma cutaneum idiopathicum hemorragicum* (*Kaposi*) the size of a pea into a rabbit. At first the piece flattened down, but at the end of two years began to grow and attained the size of a chestnut; it was adherent to the adjacent tissues. The microscopic structure was that of an endothelioma or lympho-sarcoma and differed from the original material implanted. Reale considered the deviation in structure to be due to the influence of the new environment.

A. Vischer[20] in December, 1902, injected into the peritoneal cavities of a rabbit and a guinea-pig a cubic centimeter of a mixture of melanotic sarcoma and salt solution. On February 17, 1903, the rabbit was killed and in the peritoneal cavity there were found many small deeply pigmented nodules. The peritoneum did not show a trace of inflammatory changes. Microscopically the nodes consisted of a delicate connective tissue frame work with spindle cells and a few blood vessels. In the stroma were found many large round cells filled with pigment. Giant cells were also found. Nowhere were cells that resembled those of the tumor observed either free from pigment or poor in it. All those cells without pigment were either spindle or small round cells and had no resemblance to the cells of the tumor. The pigmented nodules were in all probability the result of reaction to the presence of foreign materials. Similar appearances can be obtained by the injection of india ink. The inoculated guinea-pig showed a condition similar to that in the rabbit.

[16] Jürgens, *Verhandl. der Berlin. med. Gesellschaft,* 1895, xxvi, 119. *Verhandl. der deutsch. Gesell. fur Chirurgie,* 1896, xxv, 84; 1897, xxvi, 154. Naturforscherversam. Düsseldorf. 1898.

[17] Mayet, *Lyon médicale,* 1902, xcviii, 17. *Gazette hebdomadaire,* 1902, vii, 64.

[18] v. Bambecke, *Semaine medicale,* 1893, xiii, 8.

[19] Reale, Tentativi d'inoculazione sperimentale del sarcoma cutaneo (tipo Kaposi), Napoli, 1902.

[20] A. Vischer, *Beiträge zur klin. Chirurgie,* 1904, xlii, 617.

Gaylord[21] inoculated dogs and guinea-pigs with carcinomatous tissue. In the dog there resulted numerous small nodules in the liver which were said to be cancerous. A guinea-pig showed carcinoma nodules in the lungs. Dagonet[22] took a lymph node from a case of a recurrent squamous epithelioma of the penis, ground it in a mortar and injected two cubic centimeters of it into the peritoneal cavity of a rat. The animal became emaciated and died fifteen months later. At the autopsy several nodules were found in the omentum, a large nodule in the liver and one in the spleen. The nodules were regarded as squamous epitheliomata, the cells being smaller than those of the primary tumor.

Dagonet and Mauclaire[23] took two cubic centimeters of a mixture of rectal carcinoma and salt solution and injected it into the peritoneal cavity of a rat. The animal became emaciated and was killed in six weeks. In the peritoneum were found several small ulcerating nodules. A second rat was similarly inoculated from the first. At the end of two months the abdominal cavity contained a large lobulated growth constituting about one third of the animal's weight. The microscopic structure was identical with that of the tumor in the first rat, but they both differed from the original columnar cell carcinoma. The authors designated the growth a *sarco-carcinoma.*

Lewin in discussing the above cases believes that in all those instances where the peritoneum was concerned the tumor-growths were nothing more than the result of an inflammatory reaction, namely, granulation masses. Von Hansemann[24] states that the transplantation of tumors from man to animal has never been successful. The reported positive results are easily explained by the facility with which the peritoneum in reacting to chronic irritation forms inflammatory tumors. The tumors which Jürgens obtained were nothing else than granulomata, and Dagonet's sacro-carcinoma was a pure inflammatory growth. In the other cases there may have been an accidentally associated tumor, as in Gaylord's. The tumor in the liver may not have been positively carcinomatous, and even supposing it to be it does not prove that it was due to the inoculation, as it represented the one positive result in many attempts. The carcinoma in the lung of Gaylord's guinea-pig was, according to von Hansemann, doubtless a benign adenoma such as have often been described as arising spontaneously.

Lewin[25] reports the following experiments on the transplantation of human cancer tissue into dogs. He took portions of a very rapidly growing ovarian

[21] Gaylord, cited by v. Hansemann, *Berl. klin. Woch.*, 1905, xlii, 313, 361.
[22] Dagonet, *Compt. rendu de la Societé biologie*, 1903, lv, 966.
[23] Dagonet and Mauclaire, *Arch. de méd. exper.*, 1904, xvi, 552.
[24] v. Hansemann, *Berl. klin. Woch.*, 1905, xlii, 313, 361.
[25] Lewin, *loc. cit.*

cancer and introduced them into the abdominal cavity of a dog. Three weeks later the animal was killed with chloroform and an autopsy made. There was found in the scar of a previous operation a tumor about the size of a joint of the little finger. This was quite firm and projected into the abdominal cavity. The peritoneum, especially the omentum and the mesentery, was covered with many small nodules varying in size from a pin point to a pin head, greyish-white in color and fairly firm. Some were also found on the gall bladder and the diaphragm. No neighboring zone of redness existed, the coils of intestine were not adherent, the peritoneum was smooth and shining and there was no fluid present in the cavity. The retro-peritoneal nodes were swollen, and although quite soft showed no caseation. One small but enlarged lymph node occurred behind the manubrium.

The microscopic examination showed the following: The peritoneal nodules are composed of masses of round cells between which lie a few with lobulated nuclei and spindle cells. Besides these are seen large, pale, endothelial nuclei. Vessels are present, no necrosis, no giant cells. The retro-peritoneal lymph node had the appearance of an inflammatory swelling. The scar tumor showed crossing strands of connective tissue with round and large spindle cells and lobulated leucocytes. In places the large cells were particularly numerous. Neither necrosis nor giant cells occurred.

Two dogs were inoculated with portions from the first growth. In the third generation four dogs were employed, two in the fourth and two in the fifth. In all instances further growths were obtained. In no case however was there a resemblance to the primary tumor and the author believes that in none was he dealing with a carcinoma. The structure was not typically sarcomatous although in many places it might be considered as such. In view of the work done by Ehrlich and Apolant,[26] in which sarcomatous tumors developed in mice as a result of the transplantation of cancer tissue, it would not be very surprising if a sarcomatous condition had followed in the course of the implantation of carcinomatous structures.

The possibility of the above tumors being infectious in character was considered and many examinations were made to determine that point. No organisms could be found by staining or by the inoculation of many forms of culture media. Portions of the growth were ground in a mortar and passed through a filter coarse enough to allow the passage of bacteria. Two dogs were injected with this material with negative results, while two other dogs were successfully inoculated with untreated pieces from the same growth.

A. Sticker[27] in numerous experiments, using goats, dogs, cats, rabbits, guinea-pigs, rats, and mice and inoculating in every possible way, was unable in so much as a single instance to transmit human cancer to the lower animals. In the course of time the inoculated juices and pieces of tissue were completely absorbed. The most careful macroscopic and microscopic examination failed to reveal any tendency toward tumor formation.

Leo Loeb[28] in a series of experiments on the transplantation of tumors from one rat to another made many attempts to determine if pieces of a rat

[26] Ehrlich and Apolant, *Berl. klin. Woch.*, 1905, xlii, 871; 1906, xliii, 37.

[27] Sticker, *Zeit. für Krebsforsch.*, 1904, i, 413.

[28] Loeb, *Jour. of Med. Research*, 1901, vi, 28; 1902, viii, 44.

sarcoma could be transplanted into guinea-pigs. All the attempts were unsuccessful. Equally unsuccessful were transplantations into white mice and into hens.

M. Herzog[20] tried for several years to produce carcinoma or sarcoma in rabbits and guinea-pigs by injecting triturated and filtrated human tumor material, subcutaneously, intraperitoneally, and into the anterior chamber of the eye, but has never had any success.

Mayet[20] made numerous attempts to inoculate lower animals with a glycerine suspension of human carcinoma. He concedes that his experiments with dogs and rabbits were negative but in his experiments with fifty-three white rats he says that he certainly did four times produce "epithelial cancerous lesions" and in eight more cases probably brought about similar changes. M. Herzog[21] in discussing the above says that "One cannot read Mayet's descriptions critically without becoming convinced that he has not succeeded in producing a true carcinoma in a single instance."

In view of the previous results of the transplantation of tumors of man to the lower animals the following experiment is recorded. The results indicate that possibly under certain conditions, at present unknown, inoculation may be successfuly achieved.

On November 3, 1906, a small portion of a scirrhus carcinoma of the breast was implanted, subcutaneously, in the abdominal wall of an adult white rat, the exact age of which was unknown, but the animal was quite a large one, and presumably fully grown. At the same time a like piece was implanted similarly in a white rat about four weeks old.

The following are brief notes of the two rats.

Rat 1. Piece of tissue implanted November 3, 1906.

November 7. Animal well. Small raised area at the seat of the implantation.

November 13. Lump in abdominal wall seems slightly larger. From this date on there was no change seen in the size of the nodule. At all times it was freely movable.

April 1, 1907. The rat was found dead. For the past couple of months it had been sneezing and coughing, had lost somewhat in weight and its coat was rough and dry.

Rat 2. Tissue implanted November 3, 1906.

November 7. Animal well, small raised nodule at the seat of

[20] M. Herzog, *Jour. of Med. Research,* 1902, viii, 74.
[20] Mayet, *loc. cit.*
[21] M. Herzog, *loc. cit.*

the inoculation. No evidences of an inflammatory condition.

November 13. Nodule has been decreasing in size and can at present no longer be felt. At the present date, August, 1907, the rat is perfectly well.

An autopsy was made on rat No. 1 with the following findings: On the abdominal wall at the site of the implantation there is a small whitish nodule about 9.25 mm. in diameter. It is not ulcerated, is firmly adherent to both the skin and the abdominal muscles and does not extend to the peritoneum. No other nodules are present.

The right lung is small, firm, and contained numerous small yellowish areas of a thick, semi-caseous material. Left lung is enlarged and emphysematous. The liver contains a small parasitic cyst (tænia). Other organs are apparently normal.

The microscopical examination of the abdominal nodule is as follows:

The specimen consists of a small oval nodule about 0.5 cm. long and one-half as wide, the end being blunt. On its outer periphery it is covered by a considerable thickness of skin and subcutaneous tissue. This surface is slightly raised above the adjacent normal structures. At the ends of the oval are found a few muscle fibers, some of which seem to be degenerated and replaced by connective tissue. The deeper portion rests upon the sheath of one of the abdominal muscles.

Surrounding this nodule is a very distinct capsule of adult connective tissue, the fibers of which run parallel to the surface of the foreign mass. In this capsule are found numerous small blood vessels; they are particularly well marked along the subcutaneous periphery.

In and beneath this capsule there is found an almost continuous line of cells of two varieties. One of these contains a small round deeply staining nucleus about which is very little protoplasm; they are probably lymphocytes. The second form is a cell containing a long nucleus that varies considerably, both in thickness and in its staining capacity. Some of the nuclei are very thin and stain deeply. The larger nuclei are oval and more vesicular in character.

The long, narrow, and deep staining nuclei lie within and run parallel to the connective tissue fibers surrounding the nodule.

The fibers forming the capsule seem to consist of the pre-existing connective tissue compressed by the nodule. In a few places fibers appear to branch off from the capsule and extend a short distance into the nodule. Their identity is, however, soon lost.

Lying within the peripheral zone of the nodule and extending more or less in a right angle are many cells containing large oval or spindle-shaped vesicular nuclei. There are also found, similarly situated, numerous narrow deeply staining nuclei. In the majority of instances the nuclei are found in what appear to be interstices of the tissue.

At one place on the upper portion of the nodule there is a large area of infiltration by cells containing small round deeply staining nuclei. There are also many small newly formed capillaries in this region. Beyond the area of round cell infiltration are many oval, faintly staining, bubble-like nuclei. These have no definite arrangement, are scattered quite irregularly. Numerous collections of these larger vesicular nuclei formed of a dozen or more individuals heaped up together without any visible cell protoplasm or cell wall are also present.

The greater part of the nodule, excepting only a narrow peripheral zone, has undergone a marked degeneration. No nuclei are evident nor can any formed structures be distinguished. There are, however, certain distinct differences in the density of the tissue evident in some portions. Bands of faintly staining tissue are found extending in all directions. Between these are areas, varying greatly in shape and size, that are much less dense and which show no trace of a fibrillar structure. They appear to be filled with a distinctly granular material. In a few instances there can be seen among the granular debris small round bodies that are homogeneous and have taken a very faint bluish tinge.

On comparing this nodule with the original human specimen it is quite evident that the fibrillar bands represent the primary connective tissue, while the granular areas are the remains of the epithelial nests.

In these two cases we see that in the young rat there was within ten days a complete absorption of the implanted tissue with no further manifestations. In the older rat there was a decidedly

different course of events. The cancerous nodule was not absorbed but the bodily resistance of the rat appeared to be sufficient to prevent any increase in size or extension into other parts. The foreign tissue was walled off by a connective tissue capsule, no new blood vessels were formed and the pre-existing ones failed to obtain nourishment for the cellular structures. Consequently there was a degeneration of the epithelial elements. The connective tissue being more resistant underwent a slighter degeneration with little or no absorption.

It would, therefore, seem evident that while there was something in the younger rat that very quickly brought about the destruction of the foreign tissue, in the older rat the necessary conditions were lacking. It would therefore appear that the nodule remaining unabsorbed in this rat for five months, that a less degree of resistance of the rat, or an increased degree of virulence of the tumor might render a growth of the implanted tissue possible.

RIGOR MORTIS AND THE INFLUENCE OF CALCIUM AND MAGNESIUM SALTS UPON ITS DEVELOPMENT.*

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THE THEORIES AND SOME OF THE FACTS OF RIGOR MORTIS.

Historical.—Two features of the phenomenon of rigor mortis, the stiffness and the shortening of the skeletal muscles, bear a resemblance to two physiological processes: the clotting of the shed blood and the contraction of living muscles. Upon the basis of this resemblance two divergent theories were brought forward as far back as nearly a century ago. These theories are the ones which at the present day are still competing for supremacy.

The Contraction Theory.—Nysten(1) (1811) thought rigor to be "le dernier effort de la vie contre l'action des forces chimiques." Denuded of its vitalistic garb, the phrase expresses the view that rigor is due to a physiological contraction of the muscles. Although Brücke(2), one of the grave-diggers for the theory of vital force, rejoiced as far back as 1842, at the prospect that the theory of the "dernier effort de la vie" was then buried forever, the theory of contraction was soon resurrected, and even at the present day is occupying a well-supported position in the domain of physiology. Besides Schiff(3), Brown-Sequard(4) and some others, it was especially L. Hermann(5) and his pupils who brought together many facts in favor of the assumption that rigor mortis shows a certain form of muscular contraction.

Coagulation Theory.—As to the coagulation theory it was assumed originally by Orfila(6), Treviranus(7) and others that rigor was due directly to the clotting of the blood and lymph between the

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muscle fibers. This theory was favored also by Johannes Müller(8) for the reason that the contraction of the clot and the separation of serum from it would also explain the final release of the muscles from rigor. This theory, however, had to be given up as soon as it was recognized that rigor also occurs in completely exsanguinated animals. But Brücke offered the same theory in a modified form. Soluble fibrin, said he, enters during life into the muscle fibers and it is the clotting of this intrafibrillar fibrin which is the underlying cause of postmortem rigor. Virchow(9) called attention to the fact that the plasma obtainable from muscle differs chemically from the contents of the blood, and Brücke himself and others failed in the attempt to obtain a plasma from the muscles before they entered into a state of rigor; the chemical theory had therefore for a long period very little support from experimental facts. But in 1859 Kühne(10), who carried out all the necessary manipulations at low temperatures, finally succeeded in obtaining a liquid muscle plasma which gradually clotted spontaneously. The coagulum, which differs from the fibrin of the blood, Kühne termed myosin.

The theory that rigor is due to a sort of coagulation and resembles clotting, or in short the chemical theory of rigor, had now a solid fact for its foundation; the muscles, like the blood, while in a living state, contain a soluble proteid which clots spontaneously some time after the removal from its natural connections. That the rigor is due to the clotting of that proteid within the muscles is, of course, not a fact but only a hypothesis, but a hypothesis which is greatly supported by the following facts: that the spontaneous clotting of the myosin occurs about the same time after death as the muscles, when kept under similar conditions as the plasma, enter into rigor; that further, muscles, from which myosin is pressed out, no longer enter into rigor, and that finally the myosin obtainable from muscles already in rigor is considerably less in quantity than that obtainable from normal muscles. All these facts were brought out by Kühne himself.

Some Facts Favoring the Contraction Theory.—In the half century which passed since the establishment of the chemical theory by Kühne no new facts came to light which essentially strengthened its position, although the knowledge of the chemical nature of

myosin was considerably advanced by many investigators, especially by the extensive studies of Halliburton(11) and von Fürth(12). On the contrary, new questions arose to which the chemical theory is as yet incapable of giving a satisfactory answer. For instance, it is now well established that the clotting of blood plasma occurs under the influence of a well-defined ferment; but no ferment could be discovered, in spite of an eager search for it, which was instrumental in the clotting of muscle plasma(13). Furthermore, is it now shown that the release from rigor is not due to the setting in of putrefaction, as was believed formerly (14 and 15); the release from rigor is as spontaneous an act and as vital a phenomenon, so to say, as its onset. But the once clotted myosin fibrin and myogen fibrin, as the clotted proteid bodies from the muscle plasma are now termed by von Fürth, do not show any reversibility, do not become soluble again. Neither did the vigilant search lead to a detection of a ferment which might be concerned in the release of the muscles from rigor(13). The release reminds one rather of the final relaxation of a muscular contracture. Another fact, difficult to understand on the basis of the chemical theory, is the disappearance of rigor, at least at a certain stage, by bending or kneading the muscles. It was further established that the time of onset, the intensity and duration of rigor differ considerably between white and red muscles (14 and 16). It is known that these muscles differ in the character of their contractility. But it is not known that there is any difference in the fibrin bodies obtained from these muscles. Moreover, the onset and course of rigor depend a great deal upon the antemortem functional state of the muscles; for instance, the antemortem destruction of parts of the central nervous system(17), or cutting of the motor nerves(18), or fatigue of the muscles(19), or their paralysis by curare(20) retard strikingly the onset of rigor; while, after stimulation of motor nerves(21), or after poisoning by strychnin(22), or in deaths due to toxic tetanus(23) the development of rigor is greatly accelerated; all these are facts which indicate an intimate connection of the postmortem rigor with the antemortem muscular contractions.

Various Forms of Rigor.—The numerous studies devoted to the subject brought to light many other valuable and instructive facts,

but it would seem that this knowledge rather increased than diminished the difficulty of interpreting postmortem rigor. In the first place we have to mention other forms of rigor which are to be distinguished from rigor mortis.

1. An extremity of a living animal gets into a state of rigor if its circulation is arrested (Stenon's experiment). This form of rigor, if not too far advanced, is reversible by restoring the circulation.

2. Rigor can further be produced by submersing a muscle in water, or, still better, by injecting water into the arteries of muscles—water rigor (24).

3. By exposing muscles to higher temperatures, they pass rapidly into a state of rigor—heat rigor (25). Quite a large literature grew up on the subject of heat vigor, and there is quite a divergence of opinion as to the nature of the rigor as well as to the degrees of the temperature which cause it in cold-blooded as well as in warm-blooded animals. However, it seems quite certain that rigor occurs at a temperature which is far below the one sufficient to coagulate all the albuminous bodies. Whether there is any relation between these temperatures and the coagulation temperatures of the myosin bodies has not been satisfactorily established. It seems to us that in the literature on heat rigor the conceptions of coagulation and clotting have not always been sharply kept apart,—a confusion against which Kühne had already warned.

4. Rigor can be produced by freezing temperatures—cold rigor (Folin).[1]

[1] Folin (*American Journal of Physiology*, 1903, ix, 374) states that Brücke (*Müller's Archiv für Anatomie und Physiologie*, 1842, 176) described rigor produced by cold. But Brücke claims, against Sommer, that rigor persists after thawing and he says nowhere that freezing brings on rigor; on the contrary, he claims that rigor sets in before the freezing takes place. The single experiment mentioned by Brücke in which the amputated thigh of a frog, after being submersed in distilled water and kept in a freezing mixture, was found to be in a state of rigor after thawing, is surely insufficient evidence for a claim that rigor is produced by cold; the rigor could have been due to the distilled water—water rigor.

Folin says further that "Brücke's experiments with cold as a means of producing rigor received, curiously enough, no attention from subsequent investigators and as far as I have been able to learn the discovery has been forgotten." Folin overlooked a paper by L. Hermann (*Pflüger's Archiv*, 1871, iv, 188) en-

5. By injections of such substances as chloroform, ether, caffein-benzoate, etc. (26), directly into the muscles or intravenously, the muscles pass immediately into a state of strong rigor—chemical rigor.

Definition of Rigor.—Another puzzling question is: What constitutes rigor? Many deviations from the normal are met in rigor mortis: the muscles are stiff and of denser consistency than normal; their elasticity differs from that of normal muscles; they show an acid reaction, their irritability is lost, etc. Are all these changes essential parts of rigor and of every kind of rigor? Some believe that shortening of the muscles does not constitute an integral part of rigor. Many authors claim that the irritability of the muscle might persist even after the release from rigor (27). According to others the acid reaction is not indispensable for the development of rigor (28). Folin finally, as we have seen above, considers hardness and stiffness alone as the essential criteria of rigor.

We are apparently not yet ready for a final answer. A century of work has brought no decision between the contending theories. It has brought to light a great many single facts and that is what

titled: Die Erstarrung in Folge starker Kältegrade. This is the more regrettable as the knowledge of this paper might have been not without some influence upon Folin's method of investigation as well as upon his conclusions. As Folin did not return to this work again, as he intended to do, it might not be amiss to discuss here some of the essential points. Folin says that frog muscles which were cooled to — 15° C. were found on thawing to have gone into rigor. The rigid muscles are perfectly translucent—not opaque—and apparently are not shortened. The first important conclusion drawn by Folin from these observations is that the only fundamental characteristic of rigor is the stiffness and hardness of the muscles and that opacity and shortening of the muscles, evolution of carbon dioxide, etc., are unessential incidents of the main phenomenon. On the implicit assumption that the muscles, while they are still frozen, are already in a state of rigor, extracts were prepared from them—before thawing—and compared with extracts made from normal muscles before rigor. It was found that the spontaneous coagulation as well as the heat coagulation was the same in both extracts, and that there was no difference with regard to the degrees of acidity as well as in the amounts of the total nitrogen. Since one of the extracts was made from muscles in rigor, Folin draws the conclusion that coagulation cannot be the cause of rigor mortis. It must be remembered that this conclusion is based upon the supposition that the muscles are in a state of rigor while they are still frozen. Now Hermann says that a thoroughly frozen muscle "verfällt nach dem Aufthauen einer beschleunigten Erstarrung," that is the rigor occurs *after* thawing; the freezing has only the effect of hastening

we still need and can accomplish at present: the uncovering of some more facts—the collection of more bricks for the erection of a structure at some future time.

THE RELATIONS OF CALCIUM AND MAGNESIUM TO RIGOR

Introductory.—The aim of our paper is the contribution of a few facts regarding the relations of calcium and magnesium to the phenomenon of rigor mortis. What are these relations? On *a priori* grounds our expectations may differ with our conceptions of the nature of rigor. From the point of view of the chemical theory we might expect that the mentioned alkali earths are capable of exerting a definite influence upon the development of rigor, but that the character of the influence might be different for both substances. It is now well established that calcium salts hasten the clotting of blood. We do not know of a similar effect from magnesium salts; on the contrary, concentrated solutions of magnesium sulphate are employed to prevent or retard the clotting of blood. Do both substances behave in a similar manner towards the clotting of the muscle plasma and also towards the onset of rigor? That is, does

the onset of the rigor. Hermann says further that the thawed-out and not yet rigid muscle has a different appearance from the normal; it is translucent, and the (final) rigor of such frozen muscles differs from that of normal muscles by the *extreme degree of shortening and thickening* and by the exudation of a strongly acid serum. According to Hermann the rapid onset of the rigor does not depend upon thawing, or upon the degree and duration of the freezing, but upon the rapidity of the development of the latter. The essential points which are of interest to us here are, that according to Hermann the muscles, while they are still frozen, are not yet in a state of rigor, but that the freezing hastens greatly the onset of rigor, that the not-shortened translucent muscle is not yet in a state of rigor and that the real rigor which follows that stage is rather marked by an extreme shortening.

We offer of course no opinion as to which of the observations and views are the correct ones. But it seems to us that to uphold the conclusions of Folin the experiments ought to be repeated and the extracts ought to be made not from frozen muscles but after they were thawed out for some time, that is while they are in a state of undisputed rigor.

As to the question whether there is any difference between extracts made from muscles in rigor and from normal muscles, we may refer here to the recent investigations of Saxl (*Hofmeister's Beiträge zur Chemischen Physiologie*, 1906, ix, 1). He found that the proportion of "muscle plasma" obtainable from muscles in a state of postmortem rigor to that obtainable from fresh muscles is about 1:3.

calcium hasten the clotting of the plasma and also hasten the onset of the rigor, and does magnesium retard both? On the other hand, from the point of view of the contraction theory there are some reasons to expect that both ions might rather retard the development of rigor. It is well established, as we have mentioned above, that substances which increase muscular activity hasten the onset of rigor, while those which impair the contractions, curare, for instance, retard its onset. Now Loeb(29) has maintained that calcium exerts an inhibitory influence upon muscular contraction. A similar inhibitory influence Loeb ascribes also to the entire group of alkali earths except barium. We ourselves(30) have published several communications on the inhibitory effect of magnesium salts. But if calcium as well as magnesium is capable of inhibiting muscular contractions, rigor being in the nature of a contracture, we may expect that the effect of either ion would be in the direction of retardation of the development of rigor.

There are in the literature a few scattered statements bearing upon our question, more upon the effects of calcium than upon those of magnesium. Cavazani(31) reported that muscles poisoned by potassium oxalate do not pass into a state of rigor. As the oxalates precipitate calcium salts, Cavazani concludes from his observation that rigor is due to the presence of calcium salts in the muscles. However, neither Howell(32) nor Locke(33) were able to confirm the statement of Cavazani: muscles perfused with or kept in oxalate solutions passed into a state of rigor at least as early as the controls. From these experiments it could appear that calcium is of no importance in the development of rigor; but it must be remembered that potassium oxalate probably does not remove all the calcium from the muscles. Furthermore, Howell(34) himself found for heart muscle (strips of terrapin's heart) that a surplus of calcium chloride leads to rigor. Von Fürth(35), in studying the effect of many substances upon the clotting of muscle plasma and upon the development of rigor, found that the addition of calcium chloride, calcium nitrate or magnesium nitrate accelerated the clotting of a myogen solution, while the addition of magnesium sulphate did not exert such an influence. However, perfusion of the posterior extremities of frogs with ten per cent. solutions of calcium chloride or calcium nitrate did not hasten the onset of rigor.

A. Moore(36), working in J. Loeb's laboratory, tested the effects of various crystalloids upon rigor mortis by bathing the gastrocnemius muscles of frogs in solutions of these substances. For calcium chloride is was found that "a strong solution has at first a relaxing effect, but after a period varying from half an hour to one hour and a quarter, contraction begins, the lever reaching its maximum height in from three quarters to four hours." "In weaker solutions, $= n/2$, $= n/4$, contraction is slower in beginning and slower in setting, but as the strength of the solution is further decreased, rigor takes place more quickly." For magnesium chloride it is stated "that the effect is relaxing. It not only does not cause rigor, but actually prevents it." "Contrary to the above statement, however, rigor seems to be produced in the strength $= n/2$." For magnesium sulphate it is said that "no immediate effect was produced on the muscles immersed in the solution. A gradual rise began soon, however, indicating the approach of rigor, and the muscle assumed the usual rigor appearance. Rigor was noted in solutions stronger than $= n/4$."

In the extensive work of Overton(37) on the effect of different crystalloid solutions upon muscle and nerve the word rigor (Starre) does not occur, although frog muscles were kept in various solutions, sometimes for several days. We find, however, in the protocols (vol. 105, p. 220), that a frog muscle, kept in a solution of calcium chloride which is isosmotic with 0.7 per cent. solution of sodium chloride, about nine hours after removal from the body is "completely unirritable, shortened from thirty-eight to fifteen millimeters, opaque and very little plastic"; that can only mean that the muscle is in complete rigor about nine hours after death, which is for a frog muscle, kept at a temperature of 9° C., a very early onset.

Finally, Cushing(38) has found that the injection of Ringer's solution into the belly of a gastrocnemius muscle of a living frog may cause rigor which he ascribes to the presence of the calcium ion in the solution and compares this rigor with the calcium rigor of a strip of heart muscle described by Howell.

According to Moore, then, magnesium chloride as well as calcium chloride has more or less a relaxing effect upon the rigor of frog

muscles. These observations would seem to support, as argued above, the contraction theory of rigor. Moore, however, leans rather towards the chemical theory.

EXPERIMENTAL STUDIES BY THE WRITERS.

Method.—We made no observations upon the effect of bathing single muscles in the salt solutions. Our studies were made by injecting the solutions into living animals. The observations were made at first incidental to other experiments with magnesium and calcium salts. Injections were made into various species: dogs, cats, rabbits, guinea-pigs, rats and frogs. In the studies devoted directly to the subject of rigor mortis the most extensive observations were made upon rabbits. We experimented, however, also upon cats and frogs. In mammals the injections were made, at least in the main study, nearly exclusively intravascularly. In the frogs injections were also made into the lymph sacs. The intravascular injections in mammals were carried out by two methods: the intravenous and the intra-arterial. We shall report first the results we have obtained by intravenous injections.

Intravenous Injections of Calcium and Magnesium Salts.—The method of intravenous injection needs no special description. The solutions were permitted to run slowly into the external jugular vein from a burette arranged on the principle of a Marriotte's flask. Before beginning the infusion the animals were provided with a tracheal cannula and a cannula in the left external jugular vein. This, as well as any other operative procedure which may be mentioned later, was carried out under ether anesthesia. Usually two animals were studied at the same time, one for a magnesium and one for a calcium salt, the animals serving as controls for one another. In the magnesium animals artificial respiration had to be started pretty soon after beginning the infusion. But with artificial respiration and slow infusion a fairly good quantity can be introduced before the heart stops beating, the quantity, of course, depending upon the concentration of the solution.

In each double experiment the same molecular concentration of both salts was employed, and as nearly as possible the same quantity was infused into each animal. In the calcium animals the

respiration was rarely impaired during the entire time of the infusion, but they frequently received artificial respiration, nevertheless, in order to have similar conditions in both. The magnesium animals usually died from the infusion; the calcium animals mostly had to be killed at the end of the infusion, the animals being killed by opening the thorax. The solutions were employed as indicated above according to molecular concentrations, the weakest solution used being $= m/8$ and the strongest $= m/1$. The time elapsing between the death of the animal and the onset of rigor in the various parts of the body was taken as the main unit of comparison between the effects of the salts. The time of death is well marked, of course, by the final standstill of the heart. As to the appearance of rigor a distinction must be made between the fully developed state and its beginning, and between the rigor of the whole animal and that of the various parts. There is not the slightest doubt about the presence of rigor when it is in an advanced state, but its exact beginning is not always above dispute. Other difficulties with which one is confronted in this kind of observation are caused by variations in the time of onset of rigor in the individual animals, even under apparently exactly the same conditions, and by some variations in the order with which the rigor appears in the several parts of the body. However, the results obtained in our experiments were of such a palpable degree as to be in every instance above any doubt.

We shall now report our results, illustrating each series by abbreviated protocols of a few of the experiments.

ABBREVIATED PROTOCOLS OF SOME EXPERIMENTS.

Series I. Intravenous Infusions of the Salts in Molecular Solutions.

EXPERIMENT 1.—Magnesium chloride. Female rabbit, 1060 grams. Ether; tracheotomy; cannula in external jugular vein, connected with burette which contained magnesium chloride in $M/1$ solution.

11.55 a. m. Infusion begun. When 1.5 c.c. ran in, spontaneous respiration disappeared; started artificial respiration.

11.59 p. m. 4.5 c.c. ran in; animal dead. Thorax opened; left ventricle moderately contracted, right dilated and soft.

2.10 p. m. Left ventricle relaxed again. No stiffness in jaws, neck or legs.

3.55 p. m. Jaws locked; perhaps slight stiffness in neck; extremities still soft.

4.40 p. m. Neck stiffer; some stiffness of front and hind legs.

6.00 p. m. Neck stiff; rigidity of front and hind legs has progressed, but is far from being complete. (No further note on heart.)

The next day at 10.30 a. m. the animal is quite rigid.

In this experiment with magnesium chloride the jaws became rigid about four hours after death and at that time the rigidity of the neck just began. The rigidity of the legs began about four hours and forty minutes after death. It was not observed at what time the rigor attained its maximum, it was far from it six hours after death; it was found, however, to be complete next morning.

EXPERIMENT 2.—Magnesium sulphate. Female rabbit, 1140 grams. Same preparation as in previous experiment; burette was filled with magnesium sulphate in $M/1$ solution.

12.21 p. m. Infusion begun; artificial respiration started soon after.
12.30 p. m. Heart stopped, 6.5 c.c. infused.
2.10 p. m. No stiffness of any part of body, heart soft, moderately dilated.
3.55 p. m. Jaws locked; neck soft.
4.40 p. m. Neck soft; front legs soft; hind legs slightly stiff at hips.
6.00 p. m. Neck getting stiff; stiffness in all four legs, increased in hind legs.
Next day at 10.30 a. m. legs and neck are rigid.

In this rabbit (magnesium sulphate) the jaws were locked after about three hours and a half, while rigidity of the neck did not begin until five and a half hours after death. Also the rigidity of the legs was more retarded in this animal than in the magnesium chloride rabbit.

EXPERIMENT 3.—Calcium chloride. Female rabbit, 1200 grams. Burette filled with calcium chloride in $M/1$ solution.

12.48 p. m. Infusion begun.
12.54 p. m. Respiration good, but artificial respiration started.
12.57 p. m. Heart stopped. Infusion stopped, 5 c.c. infused.
1.10 p. m. Heart contracted, left ventricle more than right.
2.10 p. m. Neck quite stiff; rigor in hind legs, good stiffness on extension at knee.
2.45 p. m. Full rigor in hind legs.
3.55 p. m. Rigor quite marked in front legs.
4.40 p. m. Complete rigor in entire body (no note on jaws).
Next day at 10.30 a. m. the body is less stiff and the neck is soft.

In this animal (calcium rabbit) the neck was quite rigid one hour and fifteen minutes after death, and at that time the rigidity of the hind legs also was fairly established. In the front legs a marked rigidity was present about three hours after death. About four hours after death the entire rigor was complete. But next morning the release from rigor had already begun.

We shall add one more protocol of a calcium experiment, carried out with a $M/1$ solution.

EXPERIMENT 4.—Calcium chloride. Female rabbit, 2000 grams. Same preparation; right sciatic nerve cut at exit from pelvis; burette filled with calcium chloride in *M*/1 solution.

12.20 p. m. Infusion begun, given very slowly; artificial respiration started.

12.39 p. m. Convulsions appeared.

12.43 p. m. Infusion stopped, 8 c.c. infused. Artificial respiration stopped; no convulsions; heart continues beating freely for one minute. Soon after heart dilated and flabby.

1.00 p. m. Left ventricle contracted, right soft.

1.30 p. m. Neck quite rigid; jaws can just be opened with force; front legs show slight stiffness on flexion; hind legs some resistance on flexion; definite rigor on extension at hips; right leg stiffer than left.

2.00 p. m. Jaws locked; neck more stiff; marked stiffness of front legs on flexion; rigidity of hind legs increased.

5.15 p. m. Stiff as a board everywhere; right hind leg fully extended; left hind leg more flexed.

Next day at 10.00 a. m. neck is soft, jaws can be opened and the entire animal shows good relaxation—more so than another rabbit which received on the previous day only 4 c.c. of calcium chloride in *M*/1 solution.

In this (calcium) animal the rigor was everywhere fairly established about forty-five minutes after death. The maximum rigor of the entire animal was attained in four and a half hours. Next morning, however, the release from rigor had already begun and was considerably advanced.

In this series with intravenous infusion of molecular solutions the onset of rigor in the animals receiving magnesium chloride and magnesium sulphate was delayed by a few hours in comparison with the onset of rigor in the calcium animals. In the latter the onset as well as the complete development of the rigor appeared quite early and the earlier the larger the infused quantity was. The release from rigor appeared earlier in the calcium than in the magnesium animals.

Series II. Intravenous Infusions of M/2 Solutions.

EXPERIMENT 4.—Magnesium chloride. Female rabbit, 1700 grams. Preparation same as in previous experiments; burette filled with magnesium chloride in *M*/2 solution.

11.09 a. m. Infusion begun.

11.10 a. m. Respiration shallow; artificial respiration started.

11.20 a. m. 19 c.c. ran in; occasional heart beats; artificial respiration stopped; animal dead (thorax not opened).

12.42 p. m. No stiffness anywhere.

12.50 p. m. No definite stiffness.

5.00 p. m. Moderate, but definite stiffness of hind and front legs, more in hind legs.

Next day, 11.00 a. m., animal stiff; no retraction of head, and legs not extended; right ventricle of heart dilated and soft, left ventricle moderately contracted. No relaxation of body set in during the day.

Five and a half hours passed before any sign of rigor appeared. The complete rigor observed next day was of a flexion type. There was no relaxation next day.

EXPERIMENT 6.—Calcium chloride. Female rabbit, 1800 grams. Burette filled with calcium chloride in $M/2$ solution.

12.01 p. m. Infusion begun; no artificial respiration.

12.14 p. m. 19 c.c. ran in; infusion stopped. Irregular respiration; feeble heart beats; a few convulsive struggles.

12.16 p. m. No heart beats felt; dead, thorax not opened.

12.42 p. m. Definite stiffness of hind legs; front legs soft.

1.15 p. m. Definite stiffness of front legs also.

2.15 p. m. Entire animal rigid.

5.00 p. m. Animal lying with head retracted, all four legs strongly extended and with marked lordosis of back.

Next day at 11.00 a. m. the animal is in the same position, but is not so stiff; both ventricles are strongly contracted.

1.30 p. m. No longer as rigid as on previous day; especially front legs soft again.

Twenty-six minutes after death of this calcium animal definite rigor was present; the animal showed later a strong rigor in extreme extension, but the release from rigor had already begun early on the following day.

Experiments on cats with $M/2$ solutions brought out the same differences between calcium and magnesium chloride observed in rabbits: early onset, and development of rigor of an extension type with a comparatively early release, in the calcium animal, and late onset, and development of rigor of a flexion type, with a late release in the magnesium animal. The left ventricles showed the same differences as the skeletal muscles.

Series III. Intravenous Infusions of M/4 Solutions.

EXPERIMENT 7.—Magnesium chloride. Male rabbit, 1180 grams. Same preparation; burette filled with magnesium chloride in $M/4$ solution.

11.34 a. m. Infusion begun, running slowly.

11.38 a. m. 5 c.c. infused; respiratory struggles; artificial respiration started.

11.50 a. m. Stopped infuson, 26 c.c. infused; heart just perceptible; stopped artificial respiration.

11.52 a. m. Thorax opened; heart not beating.

1.00 p. m. All parts of body flaccid, heart soft.

1.55 p. m. Same.

2.55 p. m. Jaws cannot be opened; neck and extremities soft.
3.50 p. m. Same.
5.30 p. m. Rigor beginning in hind and front legs, neck still soft.
Next day all parts in rigor; unchanged the entire day.

In this animal rigor began in the jaws and muscles after three hours and in the legs after five and a half hours, while the neck was still soft. There was no relaxation next day.

EXPERIMENT 8.—Calcium chloride. Female rabbit, 1690 grams. Same preparation; burette filled with calcium chloride in $M/4$ solution.

12.09 p.m. Infusion begun.

12.23 p. m. 15 c.c. infused. Heart slightly irregular; started artificial respiration.

12.30 p. m. Stopped infusion; 26 c.c. infused; stopped artificial respiration; heart good, but no respiration—apnœa.

12.32 p. m. Breathes; opened thorax; death under signs of asphyxia.
1.25 p. m. Both ventricles contracted; body flaccid.
1.55 p. m. Jaws and neck rigid.
2.55 p. m. Marked rigidity of entire body.
3.50 p. m. Extreme rigor.
Next day at 12 m., neck and front legs are soft.

In this (calcium chloride) animal the rigor began in the jaws and the neck after one hour and twenty minutes and in the rest of the body after two hours and twenty minutes. The rigor attained its maximum, however, in three and a half hours. The release began early the following day.

EXPERIMENT 9.—Magnesium nitrate. Male rabbit, 2450 grams. Same preparation; burette filled with magnesium nitrate in $M/4$ solution.

1.08 p. m. Infusion begun.

1.18 p. m. 13 c.c. infused; respiration shallow; artificial respiration started.

1.31 p. m. 32 c.c. infused; heart quite weak; stopped infusion for three minutes.

2.00 p. m. Stopped infusion, 51 c.c. infused; stopped artificial respiration; no reaction whatsoever.

2.05 p. m. Thorax opened; heart beats; a good deal of bleeding.

2.15 p. m. Heart stopped beating; everything soft; blood clots poorly.

3.50 p.m. Jaws slightly stiff, everything else flaccid.

5.00 p. m. Jaws locked; every other part of body quite soft. Both ventricles soft; right ventricle contracts on touch.

6.00 p. m. No change.

8.30 p. m. Slight rigor in legs, not pronounced; very slight stiffness in neck, if any; left ventricle rather strongly contracted.

Next day, rabbit is very stiff and rigor is of a flexion type.

In this (magnesium nitrate) animal there was even after six hours practically no rigor in extremities and neck, the jaws however

already began to show stiffness one hour and thirty minutes after death.

EXPERIMENT 10.—Calcium nitrate. Female rabbit (weight ?). Usual preparation; burette filled with calcium nitrate in $M/4$ solution.

10.50 a.m. Infusion begun.

11.10 a. m. 15 c.c. infused so far; respiration poor; inspiratory tonus; started artificial respiration.

11.25 a. m. Stopped infusion, 25 c.c. ran in. Thorax opened, no heart beats.

11.08 a. m. Jaws locked; neck slightly stiff; moderate but definite stiffness in front legs.

12.10 p. m. Some stiffness in hind legs; left ventricle strongly contracted.

12.50 p. m. Front legs extended; stand out like sticks, hind legs very stiff, but still flexed. (Not observed any further.)

This animal received of calcium nitrate $M/4$ about one half of the quantity which the animal in the foregoing experiment received of magnesium nitrate. The contrast between the two animals was very striking. While in the magnesium animal there was no rigor even six hours after death, there was in the calcium animal a definite rigor already thirty minutes after death.

EXPERIMENT 11.—Calcium acetate. Male rabbit, 1850 grams. Same preparation; burette filled with calcium acetate in $M/4$ solution.

2.15 p. m. Infusion begun.

2.32 p.m. 10 c.c. infused; lid reflex active; respiration good, but heart cannot be felt; convulsions set in. Artificial respiration started and thorax opened. Heart beats extremely rapidly, left ventricle almost in complete tetanus; very slight diastolic relaxations. (Artificial respiration, though strong, produces no apnœa.) Six cubic centimeters more given.

2.40 p. m. Heart stopped; animal dead. Heart soon became soft.

2.50 p. m. Heart beginning to show rigor, progressing from base to apex; was completed in three minutes; right ventricle remains soft, auricles pale.

3.05 p. m. Neck, jaws and four legs in good rigor. The rapid progress could be directly perceived; the legs were seen moving, shortening, sometimes in short starts.

4.30 p. m. No change; complete rigor.

Next day at 3 p. m., the neck was soft, also jaws and legs a good deal softer.

The development of the rigor in this (calcium acetate) animal was a striking spectacle. Twenty-five minutes after death the rigor was complete, and the progress was a matter of ocular perception.

In this series the magnesium nitrate had the most delaying, and calcium acetate a most accelerating effect upon the onset and development of rigor.

Series IV. Intravenous Infusion of M/8 Solutions.

EXPERIMENT 12.—Magnesium chloride. Female rabbit, 1445 grams. Same preparation; right sciatic nerve cut; burette filled with magnesium chloride in *M*/8 solution.

11.00 a. m. Infusion begun.

11.01 a. m. Started artificial respiration.

11.05 a. m. Infusions stopped; 30 c.c. infused.

11.07 a. m. Thorax opened; heart beats occasionally.

12.50 p. m. Left ventricle slightly contracted; everything soft otherwise.

3.20 p. m. Both hind legs moderately stiff, right more than left; front legs show beginning of rigor.

4.00 p. m. Both hind legs stiff, now left more than right; front legs stiffer than before; left ventricle well contracted, right still dilated and soft.

5.30 p. m. All legs very stiff; no difference between hind legs.

Next day at 2.30 p. m., front legs are soft, hind legs stiff.

In this animal with a solution of magnesium chloride nearly isosmotic with a "physiological" sodium chloride solution, the rigor began earlier than with the more concentrated solutions—about three hours after death, attaining its maximum in about six hours and showing already in the afternoon of the next day a distinct beginning of release from rigor.

EXPERIMENT 13.—Calcium chloride. Female rabbit, 1370 grams. Same preparation; right sciatic cut; burette filled with calcium chloride in *M*/8 solution.

11.27 a. m. Infusion begun.

11.37 a. m. Stopped infusion; 30 c.c. infused; convulsions; thorax opened; heart still beating.

12.40 p. m. Neck stiff, front legs, especially right, stiff; stiffness of hind legs beginning; no difference between right and left.

12.50 p. m. Right and left ventricles strongly contracted.

1.20 p. m. Left hind leg definitely stiffer than right.

5.30 p. m. Legs in full rigor, no difference between hind legs. Next day in afternoon all legs more or less soft again.

In this (calcium) animal the onset of rigor occurred also pretty early, about one hour after death, but the maximum was not attained until about six hours after death. The onset of rigor in the right ventricle occurred unusually early, after about one hour and ten minutes.

Transfusion with Sodium Chloride.—We append here an abbreviated protocol of one of the experiments made with transfusion of sodium chloride in molecular solution. It may serve as a control experiment to the foregoing observations.

EXPERIMENT 14.—Sodium chloride. Male rabbit, 2039 grams. Same preparation; burette filled with sodium chloride in $M/1$ solution.

12.43 p. m. Infusion begun.

12.55 p. m. Stopped infusion; 7.5 c.c. infused; animal showed not the slightest reaction; heart, respiration, lid reflex, etc., unchanged.

12.57 p. m. Killed by clamping the trachea. When the convulsions due to asphyxia were over, the thorax was opened; heart beating feebly.

1.10 p. m. Heart beating slightly.

1.45 p. m. Left ventricle well contracted; everything else flaccid.

2.05 p. m. Jaws show good stiffness; some stiffness on extension at elbow and thigh.

3.20 p. m. Neck rigid; front legs stiff on extension and flexion; hind legs very rigid at hip on extension; beginning rigor in other parts.

3.50 p. m. Practically stiff all over.

Next day, 3.30 p. m., neck soft, other parts also less rigid.

In these cases the rigor set in later than in the calcium and earlier than in the magnesium animals.

The sodium chloride animals were very little affected by the transfusion, and at the end of it had to be killed. The killing was done either by clamping the trachea or by free opening of the thorax, and the asphyxia brought on the usual antemortem convulsions. Now since any kind of convulsion hastens the onset of rigor, and since magnesium animals die without convulsions, the question arose, whether it was not these convulsions which made the rigor appear earlier in the sodium chloride animals than in the magnesium rabbits. Furthermore the very same question had to be met with regard to the accelerating effect of calcium, since the calcium salts did not paralyze the animal and frequently indeed convulsions appeared either in the course of the transfusion or shortly before the death of the calcium animals. In other words, we were confronted with the question whether the difference in the time of onset of the rigor between the calcium and magnesium animals was not due simply to the fact that magnesium paralyzes the animal and calcium does not.

Experiments with Curare in Addition to the Salts.—We have therefore carried out a few experiments in which convulsions were prevented by the previous administration of curare. We shall illustrate the results by the following two protocols.

EXPERIMENT 15.—Calcium chloride; curare. Male rabbit, 1907 grams. Usual preparation; burette filled with calcium chloride in $M/1$ solution. Curarin injected until spontaneous respiration was abolished; started artificial respiration.

12 m. Infusion begun.

12.11 p. m. Heart not palpable; stopped infusion; 7.5 c.c. ran in; stopped artificial respiration; no convulsions. Thorax opened; occasionally a heart beat.

12.50 p. m. Heart moderately contracted; jaws and legs show beginning stiffness.

1.20 p. m. Jaws locked; neck rigid; legs rigid and extended.

2.45 p. m. Rigor nearly complete, of an extension type. Next day very little softening was present.

Curare, which usually retards the onset of rigor, here exerted no influence whatsoever. Although no convulsions occurred, the rigor developed as early as is usual with such a dose of a molecular solution of calcium chloride. This experiment teaches therefore in the first place that the acceleration of the onset of rigor by the transfusion of calcium salts is not due to an antemortem appearance of convulsions. But it teaches us also that the delaying effect of curare is completely wiped out by the accelerating effect of calcium.

EXPERIMENT 16.—Magnesium chloride; curare. Female rabbit, 1320 grams. The usual preparation; burette filled with magnesium chloride in *M*/1 solution. Curare given until effective; started artificial respiration.

12.35 p. m. Infusion begun; given very slowly.

12.50 p. m. Stopped infusion, 7 c.c. infused; stopped artificial respiration. No convulsions; thorax opened; no heart beats.

2.05 p. m. Everything perfectly soft.

2.45 p. m. Jaws stiff, everything else flaccid.

4.45 p. m. Except in jaws no stiffness in any part, including heart. Animal was not seen until next day at 4 p. m., when the following note was made: Some rigor of legs present, but less than in the calcium chloride rabbit.

In this and in other experiments in which magnesium salts and curare were employed at the same time, the delay in the onset of the rigor was greater than in animals which had either magnesium alone or curare alone. The retardation of rigor mortis after magnesium salts would therefore seem to be in a degree due to some other factor besides the capability of paralyzing nerve and muscle.

Experiments with Magnesium Salts and Strychnine.—That, however, the paralyzing effect of magnesium salts is an essential factor in the delay of rigor can be seen from the following two experiments, in which, besides magnesium salts, strychnine was administered.

EXPERIMENT 17.—Magnesium chloride and strychnine. Female rabbit, 1940 grams. Usual preparation; burette filled with magnesium chloride in *M*/2 solution.

2.27 p. m. Infusion begun; given slowly.

2.35 p. m. 3 c.c. infused so far; respiration slow and labored; started artificial respiration.

2.38 p. m. 9 c.c. infused so far; no corneal reflex; animal limp.

2.40 p. m. Injected intramuscularly 1.5 milligrams strychnine nitrate—more than a minimum fatal dose.

2.54 p. m. Stopped infusion; 18 c.c. infused.

2.55 p. m. Artificial respiration stopped temporarily, heart beating; artificial respiration resumed.

2.58 p. m. Stopped artificial respiration; no convulsions.

3.00 p. m. Animal dead.

3.48 p. m. and 4.25 p. m. All parts perfectly limp.

5.15 p. m. Jaws stiff now; all other parts flaccid.

Animal was not seen until next day at 10.30; then all parts were rigid.

There were no strychnine convulsions; the magnesium salts as well as the artificial respiration inhibited them, and there was apparently the same delay in rigor as seen after the administration of magnesium salts alone.

EXPERIMENT 18.—Magnesium sulphate and strychnine. Female rabbit, 1940 grams. Usual preparations; burette filled with magnesium sulphate in $M/2$ solution.

11.09 a. m. Injected subcutaneously one milligram of strychnine nitrate, 0.5 milligram per kilo being only a toxic dose.

11.16 a. m. Animal hyperesthetic.

11.19 a. m. Convulsions; started artificial respiration and began infusion of magnesium sulphate very slowly.

11.22 a. m. Convulsions stopped; no hyperesthesia.

11.24 a. m. On stopping artificial respiration tremor of chest began again; artificial respiration resumed.

11.35 a. m. No tremor anywhere.

11.42 a. m. Infusion stopped; 30 c.c. ran in; stopped artificial respiration; no convulsions or tremor followed.

11.48 a. m. Heart still beats occasionally.

12.15 a. m. Definite stiffness of hind and front legs.

12.55 a. m. Stiff as a board.

Next day at 5 p. m., there is no beginning of release from rigor.

Although this animal received a very large dose of magnesium sulphate, the rigor was not delayed by it; the onset and development were as much accelerated, as if strychnine alone had been administered. The accelerating effects of a tetanus apparently cannot be perceptibly modified by the subsequent administration of magnesium salts.

We have made a few experiments on the onset of rigor under so-called normal conditions of death, but we shall not enter into

a discussion of these experiments nor draw any conclusions from them. From our own studies and from a study of the literature we believe that every mode of killing employed by various writers, for instance, pithing, hanging (asphyxia), exsanguination, etc., has a specific relation to the onset of rigor and has to be studied separately in a longer series of observations.

Conclusions Drawn from the Infusion Experiments.—Our experiments with the intravenous infusion of calcium and magnesium salts appear to have quite firmly established the following facts. In the first place, it seems quite certain that calcium salts hasten and magnesium salts retard the onset of rigor mortis in skeletal muscles. These effects appear to increase with the quantity of the salts introduced into the circulation; the degree of the molecular concentration in which the solutions are employed seems to be of lesser importance. The accelerating effect of the calcium salts is apparently independent of the state of contraction of the muscles; there is a strong acceleration even when antemortem convulsions are prevented by means of curare. The delay due to magnesium is apparently caused, in the first place, by the paralyzing effect of these salts upon the nervous system and the muscles. Besides the paralyzing effect, the magnesium salts may, however, contain another factor which aids in a smaller degree in the delay of onset of the rigor. The delay following magnesium plus curare seemed to be greater than that following either magnesium alone or curare alone.

Another striking difference, which was frequently observed in these experiments and which deserves to be recorded, consisted in the difference of the type of rigor which followed each of the two kinds of salts. After infusion with calcium salts, especially after larger quantities, the preponderance of extension was very manifest. Even when the animals soon after death were placed in a position in which flexion prevailed, namely, with the head bent forward, the abdomen concave and all the extremities flexed, they were found later in a strong position of opisthotonus: the head strongly drawn backward with a pronounced lordosis and the extremities stretched out. The position of the magnesium animals, on the other hand, was always that in which flexion prevailed, or perhaps more correctly, the animals remained in the position in which they were placed originally.

As to the degree of rigidity which the muscles finally attained, there seemed to be no difference between calcium and magnesium, both animals were at one time or another stiff as a board, only that the maximum stiffness was attained in the magnesium animals much later than in the calcium animals.

The release from rigor appeared as a rule definitely earlier in the calcium animals than in the magnesium animals. There were only a few exceptions in which the rigor in the calcium animals lasted as long or even longer than in the magnesium animals and in these cases the quantity of calcium salts used was greater in proportion than that of the magnesium control. The earlier release from rigor in the calcium animals seems to be due to the earlier development of rigor and occurs in all cases in which the rigor for any reason sets in early (Bierfreund). We have, however, to state that on account of the high temperature of the season in which most of the experiments were carried out not all of the animals were kept until the release from rigor was completed.

The action of the salts upon rigor was caused, at least in the main, by the cations, that is, by the calcium and magnesium parts of the compounds. As regards the anions it is probable that they too exert some effect. Calcium acetate, for instance, seemed to hasten the onset of rigor more than any of the other calcium compounds. Our experiments were in this regard not sufficiently numerous and varying to permit the giving of precise data. We believe, however, that our experiments justify the general statement that the effect which the anions may have is insignificant in comparison with the effects which the cations of the salts exert upon the onset of rigor.

As regards the order in which the several parts of the body enter into rigor, the rigidity of the jaws appeared first in our experiments and usually far ahead of the stiffness of the other parts. The neck was the next part in which stiffness appeared. In a few cases, however, the neck was the last part in which the rigor set in. Of the extremities, in the majority of our experiments, the hind legs became rigid before the front legs. This differs from the so-called Nysten system, according to which the arms become rigid before the legs, For rabbits, however, our observation agrees with those of previous observers(14).

Cutting the sciatic nerves, which is said to retard the development of rigor(39), produced in our experiments no definite results. Sometimes there was a slight retardation of the onset on the side on which the nerve was cut, but nearly as often the retardation was on the opposite side. Furthermore, even in animals with both sciatic nerves intact, sometimes the right and sometimes the left leg became stiff first; the same occurred also in the front legs.

Finally we made incidentally some notes on the onset of rigor in the heart. The observations were not so extensive and precise as those made on the skeletal muscles; but they were sufficiently numerous to permit the general statement that calcium hastens and magnesium retards the development of rigor of the left ventricle. The observations upon the right ventricle were insufficient to warrant any positive statement.

Experiments with Intra-arterial Injections.—In the foregoing experiments with intravenous infusions, the salts, even when given in molecular concentration, reached the tissues in a dilute state and reached them always in company with some blood, which according to von Fürth(26), prevents the coagulation of the muscle plasma and interferes also with the development of muscle rigor. In the experiments which we are now going to record the injections were made into the arteries towards the periphery. In these experiments the muscles of that peripheral part towards which the injections were made, came into intimate contact with a more concentrated solution of the injected salts and probably also without a great admixture of blood.

As mentioned above, such experiments were already made by von Fürth. He tested a large number of substances in mammals as well as in frogs. However, as far as the substances employed in our experiments are concerned, he made only two experiments in frogs, one with calcium chloride and one with calcium nitrate; at least these are all that he records and it is not indicated that more experiments were performed than recorded. Von Fürth has seen no accelerating effects from injections with calcium salts. We have made such injections with calcium and magnesium salts in rabbits and frogs. We shall report first our experiments on rabbits.

Intra-arterial Injections in Rabbits; Method.—The animals had

morphin and ether; laparotomy was made. The abdominal aorta was ligated always beneath the renal arteries, and a cannula was tied in its peripheral end. In some experiments the inferior vena cava also was ligated, in others again the cannula was tied in one of the common iliac arteries. When the cannula was in the aorta, one of the common iliacs was clamped during an injection in order to direct the solution into one limb, using the other as a control. The injection was made with a syringe and usually quite rapidly—not less than six or seven cubic centimeters in a minute. In these experiments calcium chloride only was tested, while of the magnesium salts the chloride and the sulphate were investigated. Only $M/8$ and $M/1$ solutions were used.

In these experiments the animals usually died during the injection or soon after it, even when the inferior vena cava was clamped—a proof of the sufficiency of the collateral circulation. When the injections were made into the aorta, with one common iliac clamped, the tissues of both sides of the lumbar region were seen to be invaded by the liquid and to become blanched. Probably also the lumbar section of the spinal cord came into intimate contact with the solutions, a point which may have to be taken into consideration when analyzing the results.

We shall again illustrate our results with a few greatly abbreviated protocols.

Series I. Intra-arterial Injections into the Hind Legs of Rabbits; M/8 Solutions.

EXPERIMENT 19.—Calcium chloride. Female rabbit, 1350 grams. Preparation as stated above; abdominal aorta ligated. Cannula tied in left common iliac artery.

12.30 p. m. Injected 20 c.c. calcium chloride of $M/8$ solution into left leg.
12.34 p. m. Thorax opened, death; no immediate stiffness.
12.40 p. m. Left leg shows some resistance.
1.30 p. m. Distinct stiffness in left leg, right flaccid.
1.55 p. m. Left leg stiff in all joints, right leg soft.
3.25 p. m. and 4.20 p.m. Left leg like a board, held extended; some resistance in right leg.

In the calcium leg the rigor began about ten minutes after injection and in the control leg not till after three hours.

EXPERIMENT 20.—Magnesium sulphate. Female rabbit, 1450 grams. Usual preparation; cannula in aorta; left iliac clamped.

11.55 a. m. Injected 30 c.c. magnesium sulphate in *M*/8 solution (into right leg). Had slight convulsions after injection.

12.00 m. Thorax opened, heart still beating.

4.00 p. m. Front legs and left hind leg stiff; right leg flaccid.

The magnesium leg was flaccid, while the other parts were in full rigor.

EXPERIMENT 21.—Calcium chloride in one leg and magnesium chloride in the other. Female rabbit, 1120 grams. Usual preparation; cannula in aorta.

11.30 a. m. 13 c.c. *M*/8 magnesium chloride injected into left leg (right iliac clamped).

11.34 a. m. Struggles.

11.35 a. m. 13 c.c. *M*/8 calcium chloride injected into right leg (left iliac clamped). Thorax opened, heart still beats.

11.50 a. m. Right leg (calcium) stiffer than left.

12.40 p. m. Left leg soft; good resistance in right.

3.20 p. m. Right leg very stiff; some stiffness in left.

In this experiment the difference between the calcium and the magnesium was striking enough, but the acceleration of the rigor in the calcium leg and the delay in the magnesium leg were not so striking as in the other experiments in which larger doses were injected.

In these and other experiments of this series the calcium was distinctly accelerating and the magnesium delaying on the onset of rigor, the injected quantity being a noticeable factor. There was no perceptible effect immediately after injection.

Series II. Intra-arterial Injections of M/1 Solutions.

EXPERIMENT 22.—Calcium chloride. Male rabbit, 1410 grams. Usual preparation; cannula in aorta; left common iliac clamped.

10.30 a. m. Injected 30 c.c. *M*/1 solution (into right leg); animal died during injection.

10.33 a. m. Right leg definitely stiff at hip.

10.35 a. m. Right leg stiff at all joints.

11.30 a. m. Right leg very stiff; slight stiffness in left leg as well as in front legs.

This greatly abbreviated protocol shows that in the injected leg the rigor began two or three minutes after injection, and was not followed by any temporary relaxation, while in the other parts of the body the rigor began about one hour after death.

Out of four experiments with calcium chloride, *M*/1, only in one was there a brief relaxation intervening between the primary contraction and the final rigor.

EXPERIMENT 23.—Magnesium sulphate. Female rabbit, 1250 grams. Usual preparation; cannula in aorta; left common iliac artery clamped.

11.35 a. m. Injected (into right leg) 30 c.c. $M/1$ magnesium sulphate; animal died during injection.
11.38 a. m. Definite stiffness of right leg at hip, left leg soft.
12.05 p. m. Both legs soft at all joints.
2.00 p. m. Both hind legs soft; slight stiffness of neck and front legs.
3.30 p.m. Some stiffness at hip in both legs; more on right side.

Three minutes after the injection of magnesium sulphate the injected leg became stiff, but softened soon, to become rigid again. Rigidity occurred even later than in the other parts of the body. The same occurred in two other experiments with $M/1$ magnesium sulphate.

In two experiments with $M/1$ magnesium chloride (30 c.c.) the leg into which the injection was made became stiff soon after the injection, the stiffness developing into complete rigor without an intervening relaxation. The injected leg behaved in these experiments rather like the leg in the majority of the calcium experiments. With regard to the other parts of the body there was no difference in the behavior towards the onset of rigor between the magnesium sulphate and magnesium chloride animals. In both cases the rigor developed in the rest of the body later than in the calcium animals, but not as late as in the animals which received the magnesium salts intravenously.

While in the intravenous injections the degree of concentration of the solutions affected but little the final results, in the intra-arterial injections the degree of concentration was quite an important factor. When the salts were injected in $M/10$ solutions, there was in the intra-arterial injections the same difference between the magnesium and calcium salts as in the intravenous injections, the former uniformly delaying, the latter accelerating the onset of rigor. In either case the cation was the main factor and the anion was of very little importance. It was very different, however, when in the intra-arterial injections the salts were given in $M/1$ solution. Here the calcium chloride as well as both magnesium salts brought on a distinct stiffness almost immediately after injection. In the magnesium sulphate animal the stiffness gave way again, the real rigor developing much later. In most of the calcium animals the early stiffness went over into complete rigor without any temporary relaxation.

The same occurred also in the magnesium chloride animals. The

early stiffness in all these cases was apparently not rigor, but simply a real contraction of the muscles, caused by the stimulation of the concentrated solutions. It was neither a specific calcium nor magnesium effect.

The rapid injection of a $M/1$ solution had the effect of a so-called "salt action," an osmotic action, perhaps. And here we found that the anion is a significant factor, the magnesium chloride acted more like calcium chloride and less like the magnesium sulphate. That in the cases of both chlorides the primary stiffness passed over directly into rigor might have been due to the greater contraction which they have primarily produced and finds its analogue in the direct transition of a strychnine tetanus into rigor, the development of which, as we found above, magnesium salts cannot suppress or delay. The same conditions probably obtain in all cases of so-called chemical rigor (see von Fürth), for instance, after injection of chloroform, ether, caffein, sodium mono-brom-acetate, quinine, antipyrin, etc.; these substances cause primarily strong contractions, which hasten the onset of rigor and into which the contractions pass without any intervening relaxation. These chemical rigors are work-rigors (Arbeitsstarre) as Santesson (40) properly terms them. The rigor which follows nearly immediately after the injection of calcium and magnesium chloride belongs therefore to the group of work-rigors and two stages must be distinguished: the stage of simple tonic contraction and the stage of actual rigor. If the tonic contraction is moderate as in most of the cases when magnesium sulphate is injected in $M/1$ solution a longer or shorter intermission of relaxation follows between the stage of contraction and that of the real rigor.

From the production of this work-rigor must be distinguished the accelerating effect of the calcium salts upon the onset of rigor in the cases where only $M/8$ solutions were employed intra-arterially or when even $M/1$ solutions were injected intravenously. In these cases at least twenty or thirty minutes passed before the onset of rigor which was never preceded by a preliminary contraction. The effect here is apparently purely a chemical one confined to the cation calcium and is never caused by any anion combined with magnesium.

In the intra-arterial injections into one of the legs the effect of

the salts upon the other parts of the body was not so well pronounced as in the intravenous injections, although the entrance of the solution into the general circulation was prompt enough to prove fatal to the animal. The premature killing of the heart caused by the rapidity of the injection prevented the salts from satisfactorily reaching and saturating the tissues so as to bring them under the same degree of influence as in the slow intravenous injections with a prolonged efficient circulation.

The subcutaneous injections which were made on rabbits, guinea-pigs and rats were not numerous and we shall not speak of them in detail. It will suffice to say that the results of these observations agree with the main results obtained by the intravascular method, namely that calcium hastens and magnesium retards the onset of rigor.

Experiments on Frogs.—The injections were made in the lymph sacs and through the aorta. The rigor in frogs sets in very late after death, sometimes even as late as thirty-six hours. The interval varies with the species of frog, with their state of nutrition, and with the season of the year. Our studies were made on *Rana esculenta,* in the early spring and at the end of the summer, and some of the animals were in a rather poor state of nutrition. In all of the controls the rigor never appeared within the first twenty-four hours. As out of every twenty-four hours the animals can be under direct consecutive observation not more than ten, it is frequently impossible to note the onset of rigor if no artificial means are employed to hasten it. We made only two sets of experiments in which the animals received the injection of the salt solutions in the lymph sacs and then left them without further interference. Another drawback in the experiment is that if both frogs received the injection simultaneously, the calcium animal survived the magnesium animal by many hours, in fact, the exact time of their death could not be noted in our experiments; they were found dead in the morning. But even so, the calcium animals of our experiments were found already quite rigid, while the magnesium animals, although they died shortly after the injection in the middle of the previous day, were either still completely soft or showed only slight stiffness. We may therefore conclude even from these few, incom-

plete experiments that calcium hastens the onset of rigor in frogs. As to the delaying effect of magnesium salts these experiments gave no positive evidence.

In the experiments with transfusions through the aorta calcium chloride, magnesium chloride, magnesium sulphate and also sodium chloride were tested in $M/1$, $M/2$ and $M/10$ solutions. In the infusions with $M/1$ solutions all animals became very stiff in the course or at the end of the infusion and remained so for several days, while the control animals became rigid on the second day and were relaxed again about twenty-four hours later. The brain was destroyed in all animals. The cord was destroyed in one set and left intact in another set. In the animals with destroyed cords the rigidity was a little less strong than in the animals with cord intact. The early and persistent rigidity occurred not only in the calcium frog, but also in the animals which received magnesium sulphate, magnesium chloride or sodium chloride in $M/1$ solutions.

A similar effect was observed when the transfusions were made with $M/2$ solutions; the onset of rigidity occurred immediately after infusion and persisted for a few days and there was also the same difference in the degree of stiffness between the animals with destroyed cord and those with cords intact.

In the above mentioned two experiments of von Fürth, one with calcium chloride and one with calcium nitrate, ten cubic centimeters of a ten per cent. solution were injected through the aorta of frogs whose cord was destroyed. A ten per cent. solution of calcium chloride is nearly equal to the $M/2$ solutions which we have, among others, employed. Von Fürth thus summarizes his observations: no rigor over night—"Ueber Nacht keine Starre." On account of the divergence of our results with those of von Fürth we shall give an abbreviated protocol of one set of such experiments.

EXPERIMENT 23.—Calcium chloride infusions in the aorta of frogs. Three medium-sized frogs. Frog 1, cord intact. Frog 2, cord destroyed. Frog 3, control. Aorta connected with burette; sinus opened; all three frogs had 30 c.c. 0.6 per cent. sodium chloride solution ran through to wash out the blood. Frogs 1 and 2 had then a transfusion with 12 c.c. calcium chloride in $M/2$ solution.

3.00 p. m. Finished transfusion. Frog 1 stiff, Frog 2 moderately stiff, and Frog 3 flaccid.

4.30 p. m. No relaxation in Frogs 1 and 2; control still soft.

Next day at 12.00 m. and 6.00 p. m., Frog 1 is very stiff, Frog 2 stiff, but less than Frog 1; Frog 3, control, is moderately stiff.

There was no doubt as to the early and persistent rigor of Frog 2—comparable with von Fürth's experiment—although the rigidity was somewhat less than in the frog with intact cord. Could the difference between our experiments and that of von Fürth be due to the difference of the character of frogs? Von Fürth experimented on *Rana temporaria* and we used *Rana esculenta*. It could not be due to the previous washing out with a sodium chloride solution, as we had the same results without such a previous washing. We must again point out that no matter how thorough and extensive von Fürth's experiments otherwise were, on the particular point in question there were only two experiments and they were entirely insufficient to controvert our results which were, moreover, in complete harmony with the results which we obtained in mammals. This agreement was not disturbed by the fact that in frogs even after infusion of magnesium sulphate there was no relaxation of the primary stiffness, since the dose which we employed in frogs, considering the small size of these animals, was in proportion much greater than that employed in mammals.

The fact that in the frogs with intact cord the rigor is stronger than in frogs with destroyed cord has its explanation in that the muscle tissue alone is stimulated in the latter by the concentrated salt solutions, while, when the cord is intact, the simultaneous stimulation of the nervous mechanism increases the motor response of the muscles, which in turn leads to a stronger rigor. The relation of the intensity of the rigor to the stimulating effect of the salts upon the cord reminds one of the strong rigor which follows strychnine poisoning.

In the frogs which received infusions with $M/10$ solutions of the salt the rigor developed late in all animals, and it was difficult to decide whether there was any acceleration or delay in its onset in case of one or the other of the salts.

However, the experiments with transfusions of $M/10$ solutions, as well as experiments in which the animals received in their lymph sacs comparatively large doses of $M/1$ solutions of the salts (about four cubic centimeters for each frog), brought out distinct results

when the posterior half of the body of the frog not skinned was submersed in a sodium chloride bath, kept at a temperature of about 38° C. This method was first successfully employed by Langendorff and Gerlach(41), and later by Nagel(18). Of course, the rigor which sets in here quite early is a heat rigor. But here again the development of the rigor in the calcium animals was far ahead of that of any of the other frogs, which received either one of the magnesium salts or sodium chloride, or were simply control animals. The lower extremities of the calcium frogs pass more or less rapidly through a stage of flexion and abduction into a terminal stage of extreme extension mostly combined with abduction. In the magnesium animals the rigor appears only a little later than in the control animal and the rigid legs remain mostly in a flexed state.

These observations demonstrate that heat rigor also is greatly accelerated by the presence of calcium in the muscles of the animals. The delaying effect of magnesium is not safely established in this line of experimentation.

The experiments on frogs have confirmed the fact that calcium hastens the onset of rigor mortis, and favors also the development of heat rigor. They have also shown that concentrated solutions of calcium and magnesium salts in bloodless animals call forth an immediate contraction of the muscles which passes then without an intermediate stage into the real rigor mortis. As to the delaying effect of magnesium, the experiments on frogs brought no positive results, the failure being essentially due to the late onset of the normal rigor in the animals.

The experiments reported in the foregoing pages have shown that the relations of calcium and magnesium salts to rigor run in opposite directions, that is, calcium hastens and magnesium retards the onset of rigor. We suggested at the outset that if the contraction theory of rigor is correct, both salts ought to retard the development of rigor, that is, on the assumption that both salts exercise inhibitory effects. Can our results be considered as contributing towards a decision between the two theories, namely as being against the contraction theory and therefore in favor of the chemical theory of rigor, and can we assume that the acceleration and retardation of the rigor in our cases have their last cause in the facts established

by von Fürth that calcium salts hasten the clotting of myogen plasma and magnesium salts do not hasten it? It sounds plausible. But there is again some discrepancy between certain points in these facts and certain details in our observations. According to von Fürth(42) magnesium nitrate hastens the coagulation of the muscle plasma, while in our experiments magnesium nitrate has retarded the onset of rigor longer than any of the magnesium salts. Besides, the argument that if the contraction theory be correct, the effects of calcium and magnesium salts ought to be similar, is based, as stated above, on the assumption of the similarity of both salts with regard to their inhibitory effects. Very recent experiments, however, have demonstrated to us that the foundation of this assumption is, to say the least, insecure.

The safer course, therefore, would be not to enter for the present into a discussion of the availability of the facts revealed in this paper in support of one or the other of the theories of rigor.

SUMMARY.

Calcium salts hasten and magnesium salts retard the development of rigor mortis, that is, when these salts are administered subcutaneously or intravenously.

When injected intra-arterially, concentrated solutions of both kinds of salts cause nearly an immediate onset of a strong stiffness of the muscles which is apparently a contraction, brought on by a stimulation caused by these salts and due to osmosis. This contraction, if strong, passes over without a relaxation into a real rigor. This form of rigor may be classed as work-rigor (Arbeitsstarre).

In animals, at least in frogs, with intact cords, the early contraction and the following rigor are stronger than in animals with destroyed cord.

If $M/8$ solutions—nearly equimolecular to "physiological" solutions of sodium chloride—are used, even when injected intra-arterially, calcium salts hasten and magnesium salts retard the onset of rigor.

The hastening and retardation in this case as well as in the cases of subcutaneous and intravenous injections, are ion effects and essentially due to the cations, calcium and magnesium.

In the rigor hastened by calcium the effects of the extensor muscles mostly prevail; in the rigor following magnesium injection, on the other hand, either the flexor muscles prevail or the muscles become stiff in the original position of the animal at death.

There seems to be no difference in the degree of stiffness in the final rigor, only the onset and development of the rigor is hastened in the case of the one salt and retarded in the other.

Calcium hastens also the development of heat rigor. No positive facts were obtained with regard to the effect of magnesium upon heat vigor.

Calcium also hastens and magnesium retards the onset of rigor in the left ventricle of the heart. No definite data were gathered with regard to the effects of these salts upon the right ventricle.

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A FATAL ANÆMIA WITH ENORMOUS NUMBERS OF CIRCULATING PHAGOCYTES.[1]

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Phagocytosis of red cells, and to a lesser extent of white cells, has long been known to occur within the blood-making organs of the body in health and to a greater degree in disease. The interest of the present case is that it exemplifies the occurrence of extensive phagocytosis in the peripheral blood, involving a destruction of red cells so rapid that it may well have been the cause of the fatal anæmia which ensued. As to the cause of this phagocytosis I am altogether in doubt, but since it involved every type of leucocyte in the blood, it seems reasonable to attribute it to the presence of auto-hæm-opsonins in the serum. Details of the case are as follows:

Clinical History.—The patient was a male, Russian Jew, twenty-seven years old. He had resided one year in Boston. His family history, past history and habits were good. The present illness was from five to six months in duration. The first symptoms were palpitation and dyspnœa; five or six weeks later his feet began to swell and he coughed much, especially when lying on the right side. Examination April 20, 1907, showed a well-developed and nourished man, cyanotic and dyspnœic on the slightest exertion. The fingers were markedly clubbed.

Heart's apex was in the fifth space, one and a half inches outside the nipple line; the right border was just outside the edge of the sternum on the right. Action of the heart was regular. There was a systolic murmur at the apex and a diastolic murmur loudest at the third left costal cartilage; a systolic murmur was heard along the right side of sternum, opposite the first, second, and third ribs. The apex systolic murmur was transmitted along the ribs and also heard in the back at the angle of the left scapula. The pulmonary second sound was accentuated. Corrigan pulse was present.

The chest showed inspiratory retraction of the intercostal spaces especially near the right nipple. There was noisy breathing over most of the chest in front and over the upper part behind. Scattered moist rales were most numerous below; in the upper part of the chest were whistling rales. At the bases of both lungs posteriorly the breath sounds were nearly absent (especially on the left) and there was dulness on percussion with diminished voice sounds and fremitus.

[1] Received for publication, November 4, 1907.

The abdomen was distended and tympanitic; the liver dulness extended from a point about an inch below the rib margin to the fifth rib. The abdomen was too distended to allow palpation of the liver or spleen. No fluid was found. The patient complained of much tenderness and pain in the splenic region and a dragging sensation. The legs showed œdema extending to just below the knees. The reflexes were normal. A few enlarged glands were palpable in the groins. The patient lived thirty-four days after the date of this examination. Œdema of the extremities with double hydrothorax, great cyanosis, jugular pulsation and Cheyne-Stokes breathing gradually developed. The urine showed the usual evidences of renal congestion. The anæmia steadily progressed. A small pharyngeal abscess was opened May 8 and found to contain many polynuclear and very few mononuclear leucocytes. A blood culture was taken with negative results.

May 24 the patient died.

Obviously the patient suffered and died from aortic and mitral disease with anæmia. But the chief interest in the case is centered in the blood.

The Blood.—With the exception of two weeks, when the patient was in the Massachusetts General Hospital,[2] he was under my constant observation from March 1, 1907, to the day of his death, May 24, 1907. Cover-glass smears were taken daily and counts of the red and white corpuscles were made at various times as shown by one of the accompanying charts, which shows a very marked and increasing leucocytosis with anæmia. An early study of the blood with the warm stage revealed the fact that there was active phagocytosis of red and white cells not only by the "large lymphocytes," but also by the polynuclear cells and by every variety of leucocyte known in normal blood. The findings suggested a careful and thorough examination of the stained smears which confirmed the warm stage findings. In this case not only were the usual blood cells (polynuclear leucocytes, lymphocytes, eosinophiles and mast cells) engaged in phagocytosis, but also the myelocyte and another type of cell, similar to that which has been described as a "plasma cell."

Warm Stage Findings.—With the warm stage the "lymphocytes" appeared to be very sensitive, responding immediately to increased heat before one would suppose that the stimuli had time to reach them. Striking the slide with a pencil would disturb the cells and cause them to draw in their processes.

[2] A report of this case has been published by Dr. W. B. Bartlett, House officer at the Massachusetts General Hospital (*Boston Medical and Surgical Journal*, 1907, clvi, 629).

An attempt to stain the cells on the warm stage by putting the stain on the slide at the edge of the cover-glass so that it would run under the cover-glass, stopped all motion in the leucocytes and the red corpuscles in their rush across the field easily broke off the processes of the "lymphocytes."

I. *The Capture and Destruction of Cells by "Lymphocytes."*—I found in all the warm stage preparations many round, mononuclear, non-granular cells which on the cold slide were of the size of a lymphocyte either of the large or of the small variety. I shall call them "lymphocytes" without meaning thereby to suggest any theory of their origin.

1. *Changes in the Cell and in the Nucleus.*—On reacting to heat, the lymphocytes *spread out* so as to attain sometimes a great size—almost filling the field of an oil immersion lens with No. 3 eye-piece and processes of protoplasm came out of them, one going over three fields of the oil immersion lens (No. 3 eye-piece). The structure of the nucleus was not noticed until after the cell began to enlarge; then the nucleus also enlarged rapidly, but after it once became enlarged the nucleus rarely went back to its former size and compactness, no matter what changes took place in the protoplasm, not even when a shock was transmitted to the cell which caused it to pull in its processes and to contract its protoplasm closely around the enlarged nucleus. (Plate IV, Fig. 1, *c*, shows a lymphocyte whose protoplasm has returned to the former size while the nucleus still remains enlarged.) Some of the small lymphocytes enlarged but little under the influence of heat and captured only such cells as were in immediate contact with them.

During phagocytosis the *form of the cell* changed rapidly and continuously, new processes going out, those already out changing their position, inclusions being destroyed or more being taken in,—all during the few seconds necessary to make a rough sketch. If the smears were thick, the processes would start out as thin streams, widening when some distance away from the cell. Often the cell nucleus would break and large pieces of it would be carried out into one or more of the processes. (See Plate VII, Fig. 4, and Plate X, Fig. 1, where pieces of the nucleus can be seen in the processes of the cells.) *Later the nuclear matter was sometimes drawn*

back into the cell, which was then multinuclear. With the recognition of this fact it is perhaps unwise to speak of these cells any longer as lymphocytes, since lymphocytes have always been defined as mononuclear. But the mononuclear lymphocyte, as we ordinarily see and define it, is in fact the *resting* lymphocyte. When it is *active,* as in some cases of lymphatic leucæmia and in this case, the nuclear material is certainly divided. In view of the staining reactions of both the nucleus and the protoplasm and of the transition stages recognizable between the classic lymphocyte and the most extreme modification, the huge, multinuclear, many-tailed cells of this patient's blood — it seems best still to retain the old term "lymphocyte."

2. *The Process of Capture.*—Both red and white cells were captured and destroyed. Two methods of capture were observed. In the first a process would emerge and envelop cells immediately adjacent. In the second a process would reach out for some distance, turn as if intending to "round up" a cell lying free in its concavity, then suddenly turn again (making a double curve) and envelop cells that had floated against its convex side while it was making its first turn. Exactly this order or procedure was followed countless times and by many different cells.

3. *The Process of Destroying Leucocytes.*—The inclusions were sometimes destroyed within the processes, sometimes drawn back into the body of the cell and destroyed there. Inclusions were not always immediately destroyed. A phagocytic cell would sometimes: (*a*) destroy one inclusion at a time, or (*b*) take in several before destroying any, or (*c*) destroy one while it was capturing others.

The white cells were destroyed by lymphocytes in two ways:

(*a*) *Mechanical.*—The protoplasm of the phagocytic lymphocyte would repeatedly knead and squeeze the cells until they were broken up and their pieces scattered through the body of the phagocyte. When a polynuclear neutrophile was ingested and compressed, its nucleus always became spherical and either remained so or was later broken up into smaller spheres (Plate III, Figs. 3, *a,* and 6, *b,* and Plate IV. Fig. 4, *b*). The crushing, kneading motions went on with machine-like regularity and approximately at a steady rate.

(*b*) *Chemical* (*perhaps Enzymic*).—The captured cell sometimes

appeared to fade away, its nucleus breaking up or dissolving *in situ,* while the granules of the protoplasm gradually disappeared until nothing was left of the inclusion but a shell or "cloudy" spot in the phagocyte at the point where the captive had been (see Plate VIII, Figs. 4 and 5). The mechanical and chemical destruction of white cells by lymphocytes are shown in Plates VII and VIII.

A study of the stained specimens showed that when a polynuclear cell was well advancd in the process of being destroyed by a phagocyte (whether the phagocyte was a polynuclear cell, a lymphocyte, or an eosinophile) the nucleus and protoplasm of the captive took, with Wright's modification of Leishman's stain, a decided greenish blue color. Occasionally the nucleus but not the protoplasm was so stained.

Occasionally when the cells were closely massed, a change was found in the staining reaction of a polynuclear neutrophile which had not been ingested but was *merely in close contact* with a phagocytic cell. The change in color was the same as that noted in the polynuclear cells after having been acted upon by the cells which had captured them.

4. *Destruction of Red Cells after Phagocytosis.*—Red corpuscles were destroyed on a larger scale (and more rapidly) than were the white corpuscles. A process of the lymphocyte would often "round up" and take in as many as six erythrocytes at a time. When once engulfed the red cells were next compressed into small yellow dots or strings and finally disappeared.

5. *Escape of Captives.*—Occasionally a red cell was seen to escape after being considerably crushed, when it would resume at once its normal shape, showing the wonderful elasticity of red corpuscles.

Some of the polynuclear cells were also seen to escape after they had been crushed until the nucleus was broken up or had become circular (Plate III, Figs. 3, *a,* and 6, *b;* Plate IV, Fig. 4, *b*). Such cells showed no amœboid motion after getting free and the nuclei had lost their structure and had hyaline appearance. Thus damaged a polynuclear leucocyte came to resemble a nucleated red corpuscle in some respects but the neutrophilic granules of the polynuclear cell were still apparent.

6. *Rate of Destruction.*—One lymphocyte was seen to destroy

fifteen red corpuscles in half an hour; another destroyed twenty-seven red cells and fourteen polynuclear cells in an hour, and one watched for three hours was seen to engulf and wholly or partially destroy sixty-seven red cells and twenty-four polynuclear cells. At the end of that time the lymphocyte was still active.

Two lymphocytes were often seen engaged in destroying one captive and occasionally a cell would be seen engulfing a cell which itself had engulfed others (Plate X, Fig. 3).

II. *Phagocytosis by Polynuclear Neutrophilic Leucocytes.*—The process is quite different from that seen in mononuclear cells.

1. The polynuclear cell sends out one or more very slender string-like processes, which appear to arise from the nucleus. No matter how far these strings go out (and they sometimes traverse several microscopic fields, see Plate II, Fig. 4), little or no diminution is apparent in the size of the nucleus or in the protoplasm of the cell whence they issue. These strings next attach themselves to other cells (either red or white corpuscles) by hooking an end into the protoplasm of the captive, or by spreading out and fastening over it like the fingers of a hand.

2. Sometimes the phagocytic cell next moves away, dragging its captive along for some distance before pulling it in. Then after a pause, the string shortens and apparently recedes into the nucleus whence it came, so that the captive is gradually drawn nearer to its captor. The force exerted by the action of these strings is suggested in Plate II, Fig. 2, where a lymphocyte is seen to be pulled out of shape after being hooked by the string-like process of a polynuclear leucocyte. Often a string is seen to fasten its end in a red cell and pull it out of shape until the red has the appearance of a crenated cell.

3. When captor and captive have been brought into closer contact by the action of the "string," the protoplasm of the captor "flows" around the captive, occasionally not taking in the whole of it but nipping off a piece. (See Plate IV, Fig. 2, *b*, where a piece of the red corpuscle is nipped in this way.) In many cases a polynuclear cell takes in only a portion of a lymphocyte, owing to the great size of the latter. The polynuclear cells are always limited in stretching power (about thirty-four microns) and often seem as if they

would split, owing to the large size of the captives they try to ingest. (See Plate IV, Fig. 1, *a*, where the polynuclear cell has taken in the protoplasm of a lymphocyte.)

Effects on the Cells Ingested by Polynuclear Neutrophiles.—After a cell has been engulfed by a polynuclear leucocyte, there is no evidence of the mechanical compression so commonly exerted by phagocytic lymphocytes. Sometimes the included cells seem to be:

1. Destroyed by a chemical process; parts of the nuclei disappear, and the protoplasm "fades."

2. Sometimes the protoplasm of the captives seems to merge with that of the capturing cell, though the nuclei of the captives remain like islands, unchanged, in the protoplasm of the captor. This process seems to occur especially when the polynuclear neutrophile is itself ingested by another cell of the same type. After this last process has been gone through, the capturing cell appears to be larger than formerly.

3. The red corpuscles ingested appear to "fade," their edges first becoming dim (Plate IV, Fig. 6, *b*). They are not destroyed as rapidly as are the white cells. The lymphocytes, on the other hand, destroy the red cells more rapidly than the whites.

Possible Relation of the Lymphocytes and Polynuclear Neutrophiles to Blood-Plates.—The blood-plates were enormously increased in all the specimens of blood taken in this case. In preparations studied on the warm stage I noticed that the polynuclear cells and lymphocytes while in active amœboid motion were constantly losing small pieces of their protoplasm. These loose fragments bore very close morphological likeness to the blood-plates, as seen in film specimens taken at the same time, and a careful search of the slide failed to reveal any blood-plates differing from these broken-off pieces of protoplasm.

Amœboid Locomotion of the Polynuclear Leucocytes.—Two parts could be distinguished in the polynuclear leucocytes—a clear portion and a granular one (suggesting the endosarc and ectosarc of the ordinary pond amœba). The polynuclear cells were actively amœboid; sending out thick, rounded processes from the protoplasm, wide or narrow, for long or short distances and drawing up the rest of the cell towards the process (like a baby creeping) were the usual amœboid motions.

Presence of Atypical Leucocytes ("Plasma Cells").—Among the leucocytes active and phagocytic in this blood were seen some which corresponded fairly well to one of the types described by some writers as plasma cells. They resembled in general the large lymphocytes, but their protoplasm was intensely basophilic (bright blue with Wright's stain) and contained large unstained granules which could be identified overlying the red or white cells ingested (Plate V, Fig. 4). (I have also heard this cell described as vacuolated.) Mitosis was found in this cell as in all the other cell types of this blood (Plate V, Fig. 6).

I have several times found similar plasma cells in leucæmic blood and in one case (lymphoid leucæmia) I found it containing inclusions.

Relation of Phagocytosis to the Anæmia Seen in This Case.—In less than three months the patient's red cells fell from 5,120,000 to 1,520,000 per cubic centimeter. This progressive and fatal anæmia (shown in the accompanying chart) might very possibly be explained as a result of the great and continuous destruction of red corpuscles by the white cells. (Plate IX suggests the number destroyed.) Since one cell destroyed twenty-seven red corpuscles in an hour on the warm stage at 98° F., when the leucocyte count was 100,000 per cubic millimeter, if *all* were engaged in the process of destroying red cells *at the same rate* they could destroy all the red cells in the body within two hours. Hence if even a small proportion of the leucocytes were phagocytic, even if their average rate of ingesting cells was considerably lower than that which I observed on the warm stage at 98° F., the amount of work called for on the part of the blood-making organs would be enormous and probably overwhelming. Hence anæmia would result.

The Type of Anæmia.—The type of anæmia seemed to vary curiously from day to day; at one time the specimens suggested a secondary anæmia with low color index, small corpuscles and normoblasts; at another period I found appearances resembling pernicious anæmia with a high color index, large red cells and megaloblasts predominating over the normoblasts. March 1, when the red count was high and the white cells showed but little phagocytosis, compared with later smears, an occasional large nucleated red cell was seen.

Variations in the Counts of the Blood Taken from Different Parts of the Body.—May 17, smears taken from each ear showed large-sized red corpuscles with a high color index, polychromatophilic cells (rarely a stippled one) and erythroblasts, but the latter were decidedly more numerous in the smear from the left ear,—eleven megaloblasts and two normoblasts in a differential of two hundred white cells, while the smear from the right ear showed only two megaloblasts and one normoblast. Puzzled by this excess of erythroblasts from the left ear over those from the right, I made a red count from each ear with the following results.

Right ear: 2,740,000 per cubic millimeter (only three erythroblasts).
Left ear: 1,430,000 per cubic millimeter (thirteen erythroblasts).

I tried this experiment twice on the same day with similar results.

The number of leucocytes in specimens of blood taken from the ears, face, shoulders, arms, legs and toes varied greatly at all times. Sometimes successive drops of blood taken from a single puncture showed very different counts. The amounts of phagocytic activity also varied from day to day but phagocytic cells were always to be found in smears from any part of the body and at no time was any great search required to find cells with inclusions.

Possible Reasons for These Differences.—The variation in the number of white cells in the peripheral blood from various parts of the body and even in successive drops from the same puncture (as shown both by white counts and smear-preparations) might be partially explained by the fact that the cells showed a tendency to clump and adhere in masses. This was seen on the warm stage, in the blood counting chamber and in the stained film-specimens. As many as fifty cells were sometimes found massed together. But though this agglutination might explain in part differences in the white counts, it does not help to explain the variation in the red counts, for no such agglutination was noted among the red cells.

The variations in the total leucocyte curve are more reasonably explained, I think, as due chiefly to the *variations in the amount of phagocytosis of leucocytes by leucocytes.*

The *larger* number of the actively phagocytic lymphocytes present in the blood from the left ear (see Table I showing differential counts) might likewise explain the *smaller* number of red cells in the same specimen.

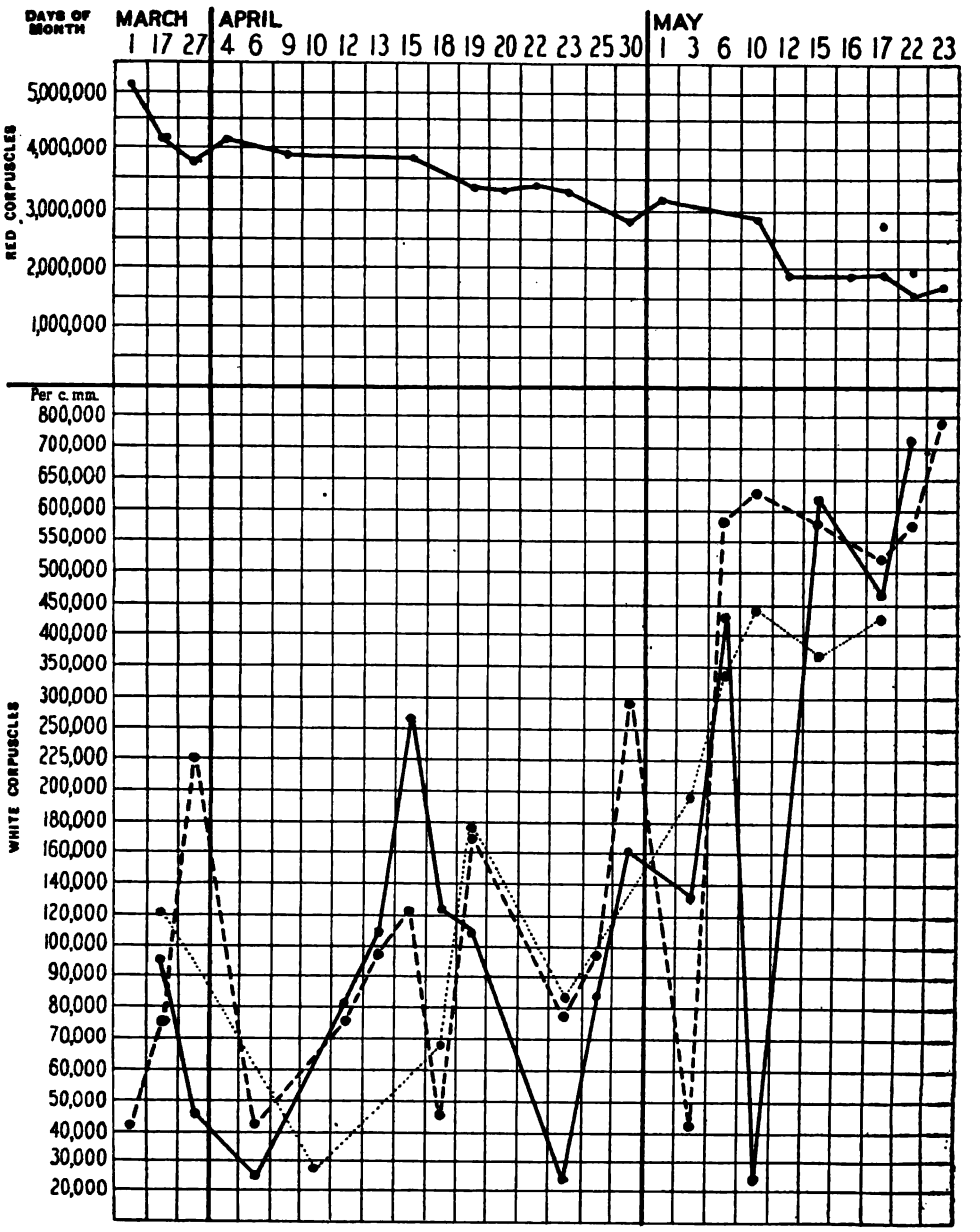


CHART I. In the lower portion of the chart the solid line —— = leucocytes from right ear; the broken line - - - - = leucocytes from left ear; the dotted line . . . . = leucocytes from extremities.

I have often seen variations in the differential counts in different smears taken from one puncture in cases of leucæmia, both lymphoid and myeloid. Whether there are similar variations in the leucocyte counts made with the Thoma-Zeiss apparatus, I do not know; but I hope to work this point out later.

The Course of the Leucocyte Curves in This Case.—In Charts I and II, and Table I, I have collected the data regarding the leucocytes in this case. In Chart I, the counts from the right ear, from the left and from the extremities are separately plotted. This chart

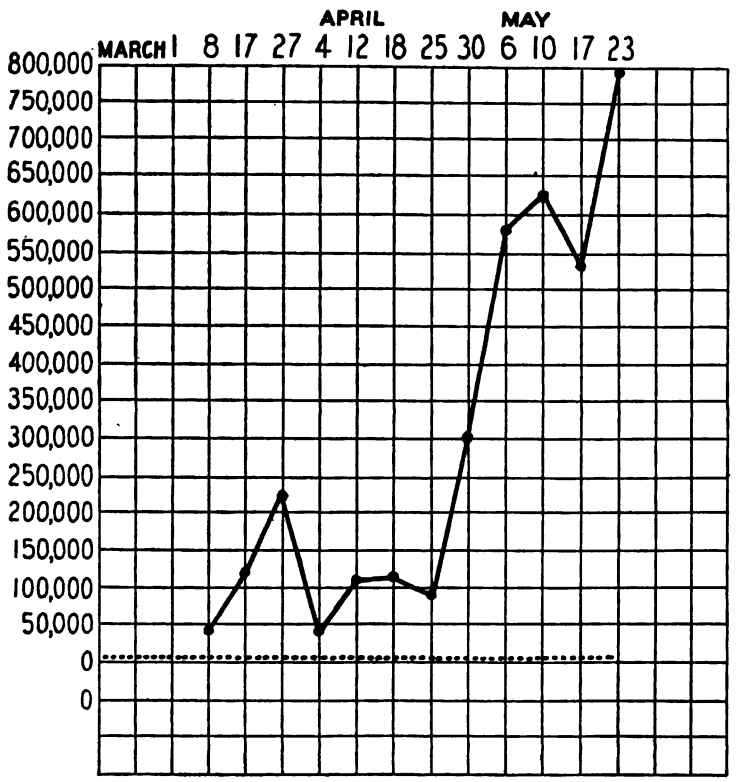


CHART II. Highest counts taken at (nearly) weekly intervals.

shows that there was on the whole an increase in the circulating leucocytes from the 43,000 found March 1 to the 700,000 present May 22. It will be noticed that the last three counts from the ears (May 15, 17 and 22) all show more than 450,000 leucocytes.

In Chart II, I have taken the highest counts only as estimated in (approximately) weekly examinations and plotted the chart accordingly on the theory that the high peripheral counts are more likely to be representative of the whole blood than are the low counts, because the low counts are likely to result in part from massing of the leucocytes or from the phagocytosis of leucocytes by leucocytes.

TABLE I.

	Differential Count of 200 White Corpuscles.							Found in Count of 200 White Corpuscles.	
Dates.	Polynuclear Cells.	Large Lymphocytes.	Small Lymphocytes.	Eosinophiles.	Mast Cells.	Myelocytes	Plasma Cells.	Megaloblasts.	Normoblasts.
March 1	63.	19.	11.	3.	2.5		1.5	1	
March 17	56.5	13.	29.5	1.					
March 22	64.5	17.	18.5						
March 30	56.5	24.5	11.5	5.	2.5				
April 6	64.	22.	14.						
April 9	63.	30.	1.5	2.5	2.		1.		
April 19	77.5	21.5		5.	.5				
April 23 (Rt.)	77.5	22.					.5		
" " (Lft.)	46.5	32.	21.5						1
April 30 (Rt.)	68.	27.5	2.	2.	.5				
" " (Lft.)	47.	32.	21.						
May 5	86.	7.5	1.				5.5	3	1
May 6 (Rt.)	62.	22.	1.5	1.					
" " (Rt.)	69.	26.	3.		2.				
" " (Lft.)	66.	27.5		2 5	2.		2.		
" " (Lft.)	68.5	28.	5.	1.5	1.5		.5		
May 7	68.	25.5		1.5	3.	.5	1.5	1	1
May 15 (Rt.)	66.5	25.5	3.5	1.5	1.	1.	1.	3	1
" " (Lft.)	48.5	43.	4.5	.5	.5	2.	1.	6	2
" " (Foot)	51.	47.5	1.5					1	1
May 16 (Rt.)	61.5	33.	4.	1.	.5				
" " (Lft.)	48.	48.5	2.5			.5	.5	4	2
May 17 (Rt.)	63.	35.	1.			.5	.5	2	1
" " (Lft.)	58.	38.5		.5	1.		2.	11	2
" " (Finger)	37.	54.5		5.5	2.5		.5	1	1
May 23 (Rt.)	65.5	32.5	5.		1.5			1	
" " (Lft.)	64.5	32.	2.5	.5	.5			1	1

The differential counts (see Table I) are difficult to interpret. Except on one day, smears taken from the right ear showed always a much higher percentage of polynuclear cells than did the smears from the left ear, while the mononuclear cells were correspondingly more numerous on the left than on the right side. The percentage and absolute number of large phagocytic "lymphocytes" increased as the case progressed and the number of small lymphocytes diminished.

Eosinophiles varied from zero to	23,000 per cu.mm.	(May 17.)
Mast cells varied from zero to	11,600 per cu.mm.	(May 6.)
Myelocytes varied from zero to	11,500 per cu.mm.	(May 5.)
"Plasma cells" varied from zero to	10,400 per cu.mm.	(May 17.)
Megaloblasts varied from zero to	28,000 per cu.mm.	(May 17.)

Effect of the Patient's Blood in Stimulating Phagocytosis in Other Human Blood.—One platinum loop full of the patient's blood was mixed with about ten loops of blood taken from a healthy adult, whose blood had previously been examined and found to be free from phagocytosis. The two were mixed upon a cold glass slide, covered with two cover slips and put upon the warm stage at 98° F. On immediate examination very little phagocytosis was observable and the experiment was at first considered negative, but an hour later a very much larger number of cells were found to be phagocytic and finally all the mononuclear cells of the mixed blood showed phagocytic activity.

Since not all of these mononuclear cells can have come from the patient's blood a part at least of the phagocytic activity of the mixed blood must be attributed to a stimulus exerted upon the normal leuccocytes by the presence of substances (perhaps opsonins) in the patient's serum.

Animal Experiment.—In the last week of April, 1907, five drops of the patient's blood was diluted with about a drachm of normal salt solution and introduced into the subcutaneous tissue of a guinea-pig's abdomen. The blood of this animal had previously been examined carefully and found to contain no evidences of phagocytosis.

About two weeks after the introduction of the patient's blood into the guinea-pig specimens taken from the animal's ear vein showed that approximately two thirds of all the mononuclear cells were engaged in phagocytosis. The cells included by the phagocytes were mostly mononuclear leucocytes, but one polynuclear cell and a few red corpuscles were also ingested.

Two weeks after this or four weeks from the time when the patient's blood was first introduced into the guinea-pig, the blood from the animal's ear vein showed that phagocytosis was still going on, though only about one half as many cells as formerly were now found to contain inclusions.

Two months after this, or three months from the time of the original injection, the animal's blood was again examined. Phagocytosis had then almost entirely ceased. The animal was killed and autopsied but nothing abnormal found.

The Extraordinary Vitality of the Phagocytic Leucocytes in This Case.—When removed from the body the leucocytes from this case preserved their powers of motion and phagocytosis for weeks and even for months as is shown by the following experiments:

1. April 20, 1907.—A drop of this patient's blood was drawn into the Thoma Zeiss pipette and then diluted with 0.5 per cent. acetic acid. May 10 an examination of a drop of this diluted blood (which had remained for nearly three weeks in the bulb of the pipette) showed that the leucocytes were still amœboid, phagocytic and able to send out processes reaching across two squares of the counting chamber.

2. April 22.—Examination of the patient's blood showed 3,290,000 red cells and 6,800 leucocytes per cubic millimeter. The diluted blood was then left (sealed up) in the pipettes. May 18 (or nearly a month later) the contents of the pipettes was again examined with the following results: Red cells 9,000, leucocytes 5,400.

There was then added to the remaining portion of the diluted blood a small amount of normal blood, so that the figures were increased to: Red cells 2,684,000, white cells 72,000.

Eight hours later this mixture was again examined. No locomotion and no evidences of phagocytosis could then be seen, but twenty-four hours later (thirty-two hours since the addition of normal blood) another examination showed that motion and phagocytosis had begun again and the cells were decidedly active.

August 22, 1907.—I examined a specimen of the patient's blood which had been taken in May, diluted (as usual) with Gower's solution and preserved in the bulb of the pipette. Even at this time, that is, after more than three months, the leucocytes were found to be still motile and phagocytic.

Possible Explanations for the Phenomena Here Described.—When the blood of this case was first examined attention was concentrated on the unusual appearances of the large phagocytic cells. What were they and whence did they arise? Were they parasites?

Were they bone-marrow giant-cells? What was the reason of their appearance?

Further study of the blood, especially with the warm stage brought out three important facts:

1. A large portion of the protoplasm of the strange cells was made up of other cells which they had ingested.

2. When free from such inclusions the majority of the phagocytic cells had essentially the characteristics of some of the "large lymphocytes" (or large, mononuclear, non-granular cells), which are seen in large numbers, for example, in malaria and in small numbers in normal blood. Every transition was found in the patient's blood between the ordinary large lymphocyte and the huge branching phagocytic cells.

3. Most important, however, was the discovery that not only the "large lymphocytes" but all the familiar leucocytes, *i. e.,* the polynuclear neutrophiles, eosinophiles, mast cells were in this case engaged in phagocytosis, though no cell type was as active as the "large lymphocyte."

This last fact suggested inevitably the inference that the presence of the enormous multitude of phagocytic cells here described was the result not of an invasion of the blood stream by foreign cells, but of changes in the serum which either (*a*) *opsonized* the red and the white cells and rendered them savory morsels for the phagocytes, or (*b*) stimulated the ordinarily feeble phagocytic powers possessed by all the leucocytes of the blood, or (*c*) produced both these results.

The presence of *auto-opsonins* or *auto-hæm-opsonins* in the patient's blood would explain all the facts of the case.

A glance at the table of differential counts on page 89 shows that the different varieties of leucocytes were present in essentially normal percentages, or, in other words, that the stimulus leading to the increase (and presumably to the phagocytic activity) of the cells was exerted on *all* the leucocytes and not on any single variety. But the presence of myelocytes and other abnormal varieties (plasma cells) suggests that we have to do in this case not merely with an aggregation of leucocytes in the peripheral blood but with a new formation in the blood-making tissues (leucæmia). Phagocytosis was performed by these abnormal varieties—myelocytes and plasma

cells—as well as by the normal varieties of leucocytes. (A plasma cell is shown in Plate V, Fig. 4, acting as a phagocyte.)

The auto-hæm-opsonic theory here advanced as an explanation of the facts in this case is further supported by the human and animal experiments which have been described.

This case and that reported by Dr. VanNuys,[3] which I believe to be practically identical with this one, are the *most marked* instances of peripheral intravascular phagocytosis; but for several years I have occasionally recognized in the peripheral blood of ordinary cases of leucæmia—both myeloid and lymphoid—phagocytosis of other blood cells performed by each of the types of leucocytes. I hope to work up and report more fully on this matter in a later paper.

No autopsy was allowed but punctures were made into the spleen, the liver, the enlarged inguinal glands and the lungs.

In the blood drawn from the spleen I found essentially the conditions described in the peripheral blood. There were also a good many giant cells from twenty to thirty microns in diameter and containing many nuclei (five to seven), sometimes peripherally arranged (Plate X, Fig. 5). To my surprise not one of the forty or fifty giant cells examined showed any inclusions or other evidence of phagocytosis. The different varieties of leucocytes already described as phagocytic in the peripheral blood were also phagocytic in the spleen.

In the blood from the lungs polynuclear leucocytes were much more numerous than in the peripheral blood and often contained cocci. The smears from liver and lymph glands showed nothing remarkable.

I wish to express my gratitude for constant assistance and advice to Dr. Stuart J. Lawson, to whom I owe the micro-photographs for this article. I have also been greatly aided by the encouragement and advice of Dr. Eugene L. Opie, Dr. Ludwig Hektoen and Dr. Mark W. Richardson; by Dr. G. G. Sears, who kindly allowed me to study the case in his wards at the Boston City Hospital, and by Dr. Richard C. Cabot, who has studied many of my specimens and verified my findings. To all of these gentlemen I wish to express my sincere thanks.

[3] VanNuys, *Boston Medical and Surgical Journal*, 1907, clvi, 390.

EXPLANATION OF PLATES.

Plate I.

Fig. 1. (*a* and *b*) × 1530. Polynuclear leucocytes with phagocytic processes starting out from the nuclei and crossing adjacent cells. Note in Fig. 1 (*a* and *b*) and in Fig. 2, the gourd-shaped enlargement at the extremities of the narrow process.

Fig. 2. (*a* and *b*) × 765. Shows a polynuclear cell with a process extending across an entire field. At the right of the field is a typical phagocytic "lymphocyte."

Fig. 3. × 765. A process from a polynuclear cell showing division into several strands.

Fig. 4. × 675. A polynuclear cell with two phagocytic processes, the longer one is attached to a red corpuscle, the other (and shorter process) splits at the end and encloses a polynuclear cell like the fingers of a hand.

Plate II.

Fig. 1. × 765. Shows a polynuclear leucocyte (distorted and torn by technique) with its process attached to and constricting a portion of the protoplasm of a lymphocyte (the arrow points to the constriction).

Fig. 2. ×1215. Shows at the top of the figure a polynuclear cell with phagocytic process attached to a lymphocyte.

Fig. 3. ×1530. Shows a process coming from a polynuclear cell in the upper left-hand portion of the figure; the process is composed of several strands. Some of them are torn, some turn and go under, but do not touch the lymphocyte which is just below the polynuclear cell.

Fig. 4. × 225. Shows the latter polynuclear cell on the extreme left and gives some idea of the length of some of the processes. The strand is broken in one place, but one of its threads remains intact and connects with the point on the right of the field where the string is again seen distinctly. The very marked increase of leucocytes can be estimated from this field.

Plate III.

Fig. 1. × 1125. A leucocyte in mitosis. With Wright's modification of Leishman's stain, the protoplasm shows the granulations of the size and color characteristic of the polynuclear leucocyte and the structure of the nucleus differs somewhat from that of any of the lymphocytes or myelocytes seen in mitosis.

Fig. 2. × 1125. One of the giant polynuclear cells, quite commonly seen in this case; when measured with a micrometer these giant polynuclear cells were found to vary very little in size. All were approximately 34 μ in diameter.

Fig. 3. (*a* and *b*) × 1125. (*a*) A polynuclear cell which has escaped after being "mauled" by a phagocyte. (*b*) A polynuclear cell showing a loosened structure and a loop in the nucleus similar to that seen on the warm stage when the nucleus was about to send out processes as the first step in phagocytosis.

Fig. 4. × 1530. (*a*) Polynuclear cell showing the amœboid processes used in locomotion (not phagocytosis). (*b*) × 1125. Pieces of protoplasm probably broken off from a polynuclear cell in the process of locomotion.

Fig. 5. × 1530 and 1125. Shows various forms taken by the polynuclear cells in the process of amœboid motion.

FIG. 6. × 1125. (*a*) A polynuclear cell with a red corpuscle included. (*b*) An "escaped" polynuclear cell whose nucleus has become circular as a result of compression exerted by a phagocyte. These "escaped" cells when watched on the warm stage showed no amœboid motion after their "escape." (*c*) Lymphocyte in mitosis.

PLATE IV.

FIG. 1. × 1530. (*a*) A polynuclear cell with the protoplasm (but not the nucleus) of a lymphocyte enclosed. The polynuclear cell shows the stretched, split appearance often seen as the result of attempts to take in an inclusion too large to be completely engulfed. (*b*) A polynuclear cell with another polynuclear partly taken in. (*c*) A phagocytic "lymphocyte" showing the nucleus still spread out so that its structure can be easily seen though the protoplasm has retracted to the usual size. Notice how tightly the nucleus appears to be stretched "around" the protoplasm. The protoplasm retracts but the nucleus seldom does.

FIG. 2. × 1125. (*a*) A polynuclear cell with a red corpuscle enclosed in an "arm" which is indicated by dark dots at the rim of the corpuscle. (*b*) × 1125 A polynuclear cell enclosing a red corpuscle, a piece of which was nipped off when the protoplasm of the polynuclear closed around it.

FIG. 3. × 1125. Shows two polynuclear cells each engaged in engulfing several red corpuscles.

FIG. 4. × 1530. (*a*) A polynuclear leucocyte with another polynuclear cell ingested and partially destroyed. (*b*) This figure shows the appearance of a polynuclear which has escaped after being partly destroyed.

FIG. 5. × 1530. (*a*) A polynuclear leucocyte showing two compartments which contain the remains of former captives. (*b*) A polynuclear leucocyte with another polynuclear cell partly ingested.

FIG. 6. (*a*) × 1125. A polynuclear leucocyte whose protoplasm has surrounded a red corpuscle. (*b*) × 1530. A polynuclear leucocyte with a red corpuscle ingested. (*c*) A lymphocyte with an inclusion.

PLATE V.

FIG. 1. × 1125. An eosinophile with a red corpuscle ingested. Below this is a polynuclear neutrophile.

FIG. 2. × 1530. An eosinophile with a polynuclear cell partially ingested. The part that is being acted upon by the enzyme of the eosinophile is stained blue while the part that is outside still retains the normal staining reaction of the polynuclear cell.

FIG. 3. × 1530. An eosinophile with two polynuclear cells ingested (the eosinophile granules do not show well in the photograph).

FIG. 4. × 1530. A cell (with similar appearance to a cell described by some observers as a plasma cell) whose so-called "vacuoles" are opaque and do not stain with Wright's modification of Leishman's stain, as can be seen by noting that some of them overlie the red corpuscle which the cell has ingested. I refer to this cell in the differential counts as a "plasma cell."

FIG. 5. × 1530. The "plasma cell" in mitosis.

FIG. 6. × 1530. A similar cell taken from a smear from the patient's spleen.

PLATE VI.

FIG. 1. × 1530. (*a*) A mast cell with a phagocytic process extended. (*b*) A mast cell with an inclusion.

FIG. 2. (*a*) × 1125. A small lymphocyte showing a "digestive vacuole" and a long protoplasmic process. (*b*) × 1530. An amœboid myelocyte.

PLATE VII.

Mechanical destruction of cells by the lymphocytes.

FIG. 1. × 1530. A lymphocyte capturing and constricting a polynuclear cell in its crescent-shaped "claw."

FIG. 2. × 1530. A lymphocyte with two polynuclear cells engulfed.

FIG. 3. × 1530. Shows a lymphocyte whose "digestive vacuole" contains a polynuclear cell which has been much compressed by the action of the phagocyte. The amount of compression and the force exerted can be estimated by comparing the size of the captive with that of the surrounding red corpuscles.

FIG. 4. × 1125. A lymphocyte with two processes extended; each process contains a piece of the cell-nucleus. Notice the blood plates assembled at the ends of each process. The lymphocyte has ingested two polynuclear leucocytes and compressed them until their nuclei are circular.

FIG. 6. × 810. Shows a single enormous lymphocyte full of inclusions of various kinds. The single loose structured nucleus of the including cell is visible near the center. An including process of protoplasm can be traced at the extreme left. This cell was found in a smear taken at a time when all the cells were especially active in phagocytosis.

PLATE VIII.

Chemical destruction of cells (perhaps by enzyme).

FIG. 1. × 1125. A lymphocyte with three processes of protoplasm extended in preparation for phagocytosis.

FIG. 2. × 1530. A lymphocyte containing two polynuclear cells in its "digestive vacuoles." (*a*) Shows the structure of the nucleus of the polynuclear cell just disappearing. (*b*) Shows a change in the structure of both the nucleus and protoplasm of the polynuclear cell.

FIG. 3. × 1530. Shows very distinctly (on the left) a polynuclear cell in the process of being destroyed.

FIG. 4. × 1530. A lymphocyte whose digestive vacuole contains but a bluish-stained mass, probably the remains of a polynuclear cell.

FIG. 5. × 1530. A lymphocyte whose digestive vacuole contains only a shell of the inclusion.

PLATE IX.

Extensive destruction of red corpuscles.

FIG. 1. × 1125. A huge multinuclear "lymphocyte" with two red corpuscles ingested and its process in the act of capturing another.

FIG. 2. (*a*) × 1125. A lymphocyte packed with captured red corpuscles and a process out at the upper end. (*b*) × 450. Shows a polynuclear cell caught at one end; below is a process of protoplasm surrounding more cells.

FIG. 3. × 1530. Cell with nine red corpuscles included.

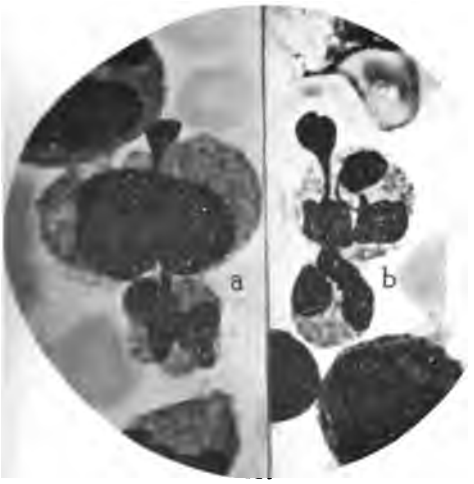
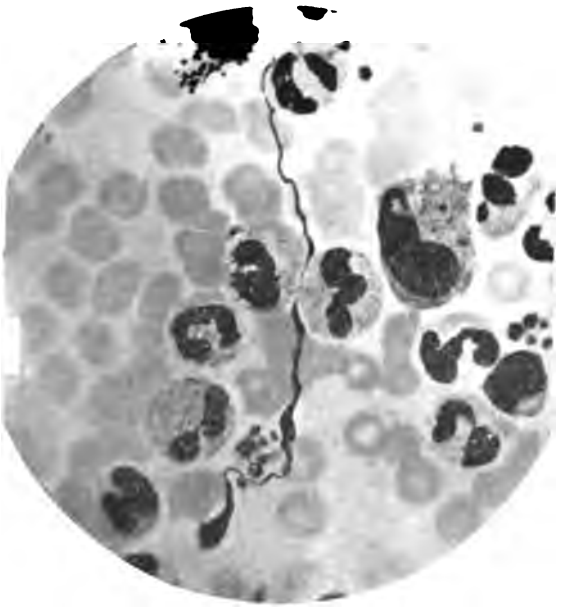


Fig. 1.



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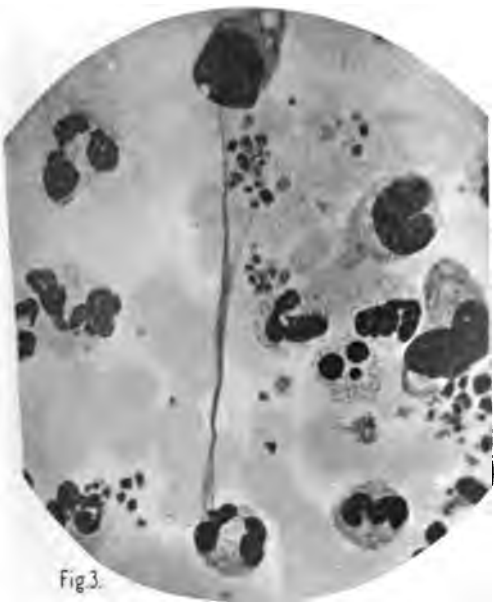

Fig. 3.

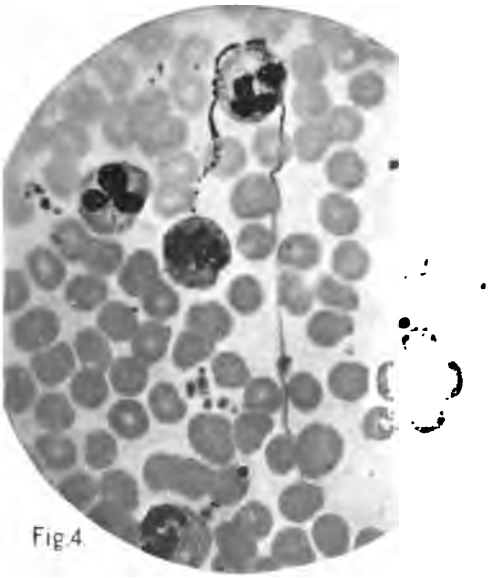

Fig. 4.

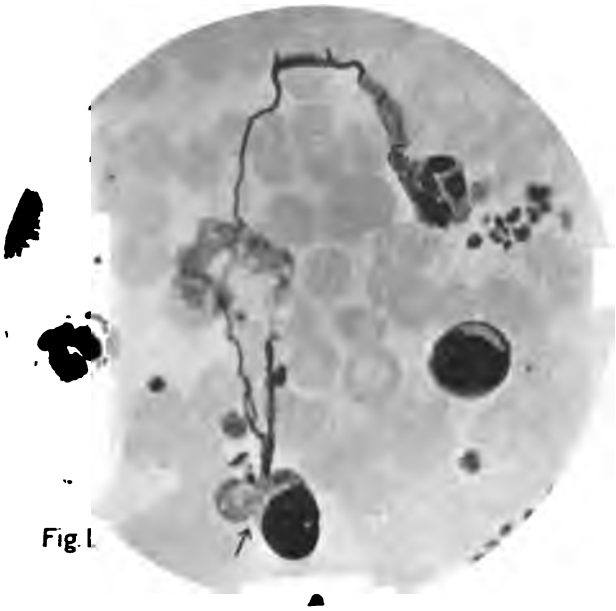

Fig. 1.

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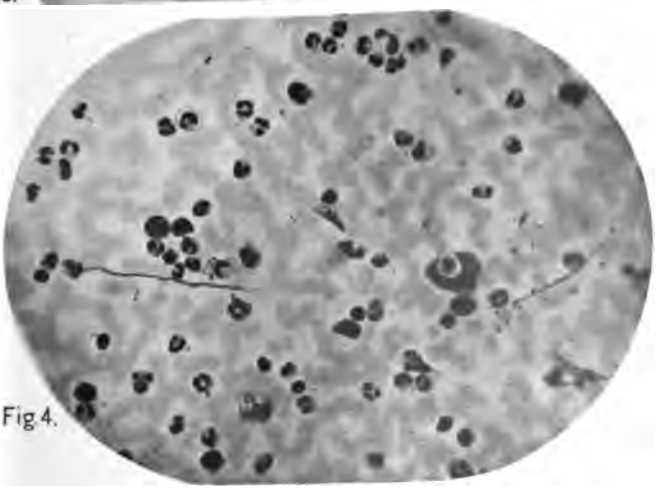

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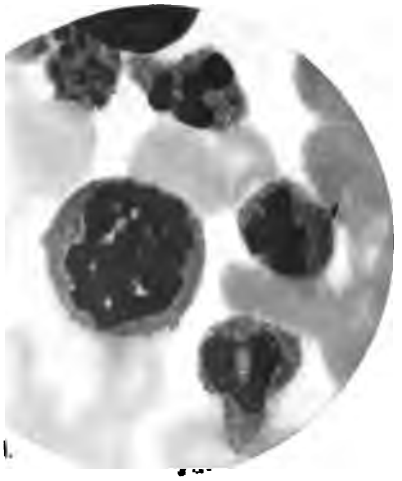

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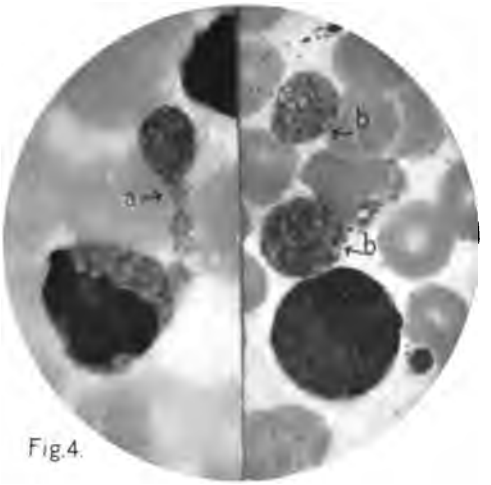

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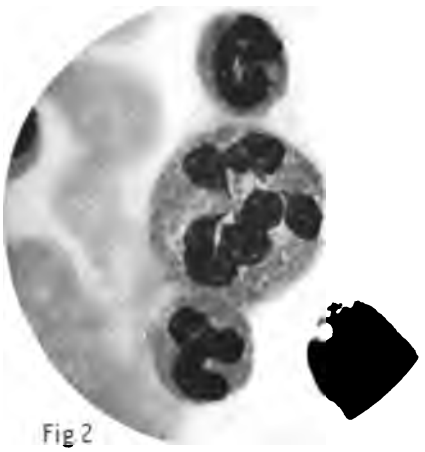
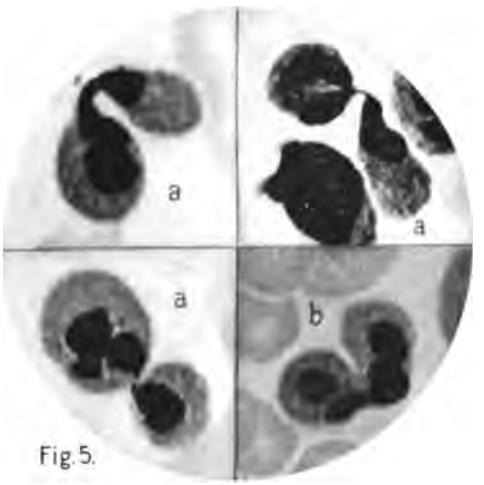


Fig 2



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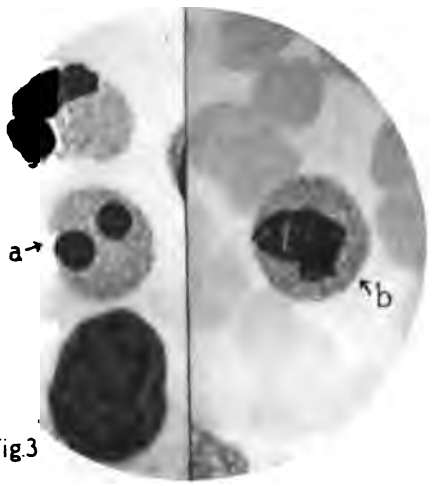


Fig. 3

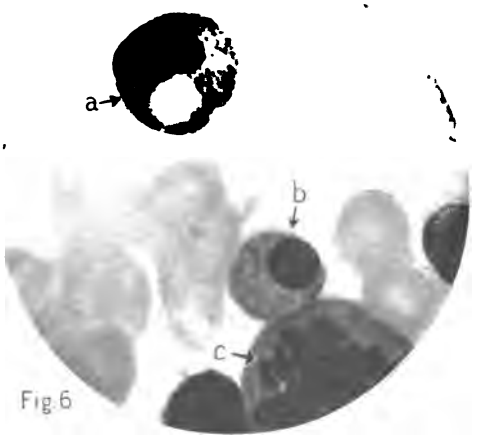
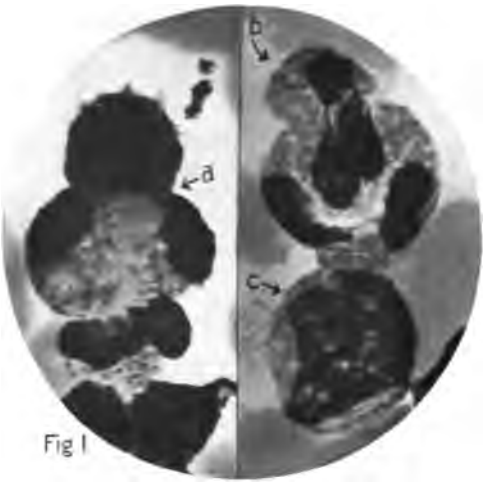


Fig. 6



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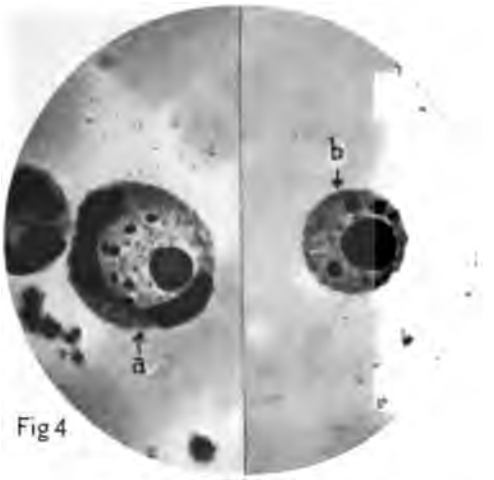

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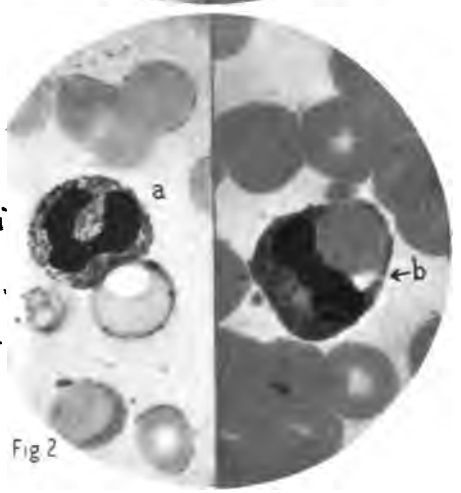

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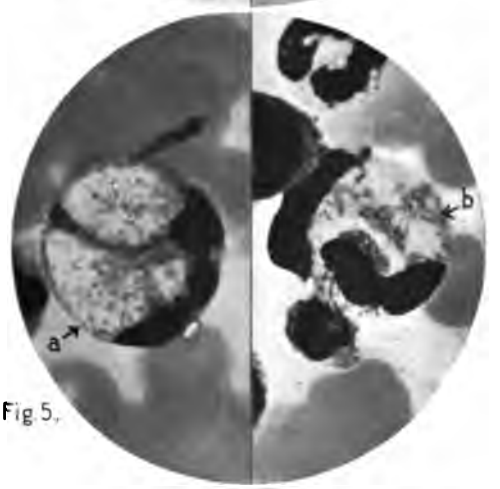

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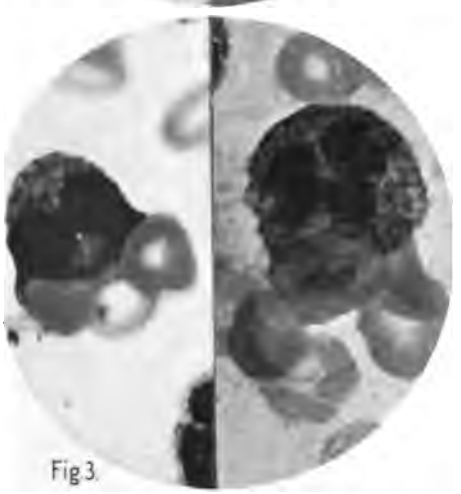
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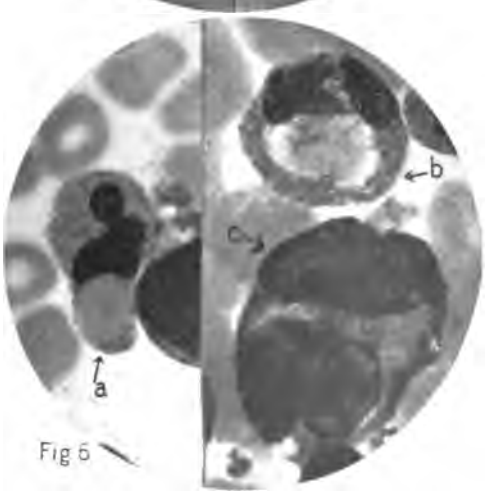
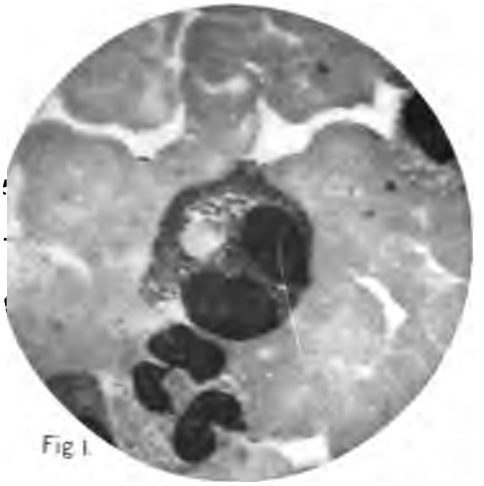


Fig 6



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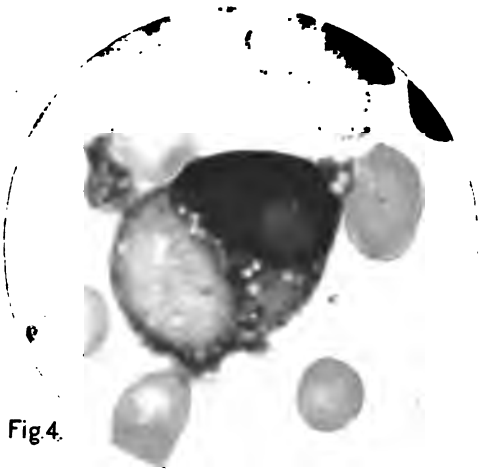

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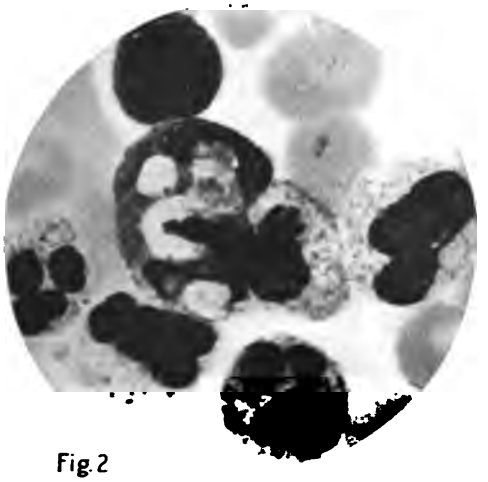

Fig. 2

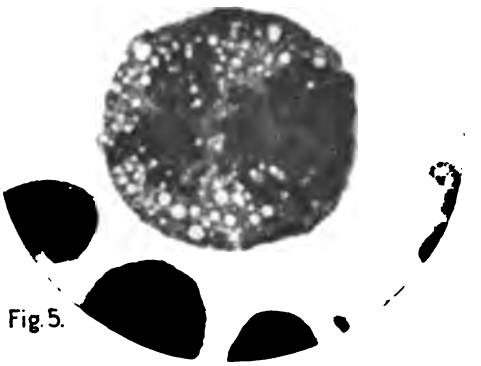

Fig. 5.

Fig. 3

Fig. 6.

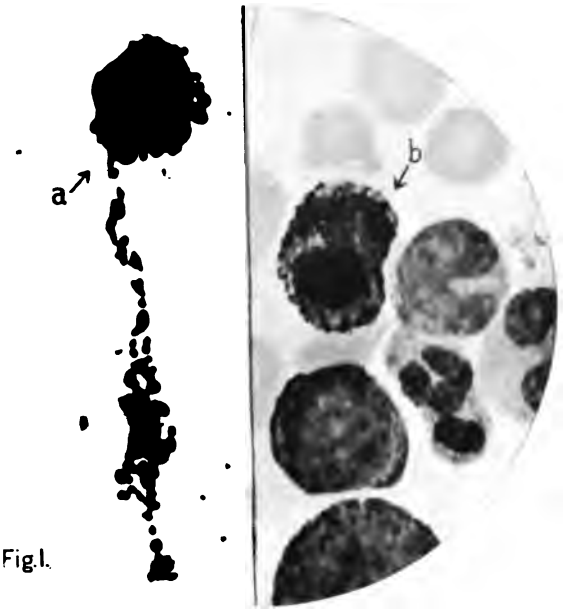

Fig. I.

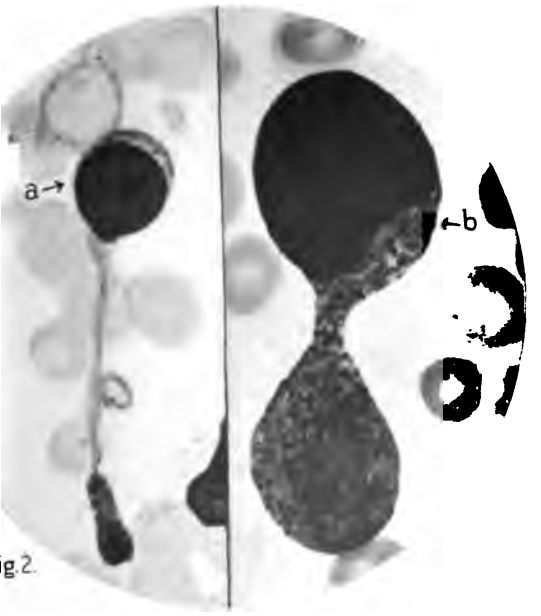

Fig. 2.

Fig. I.

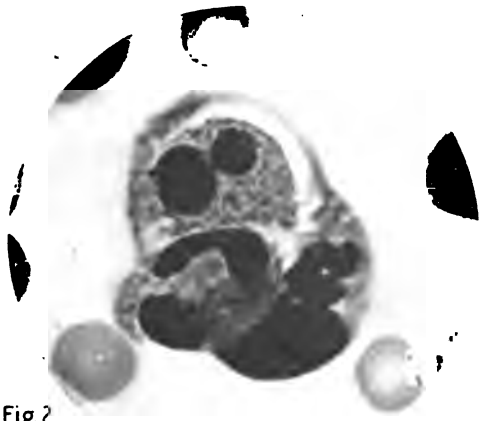

Fig. 2.

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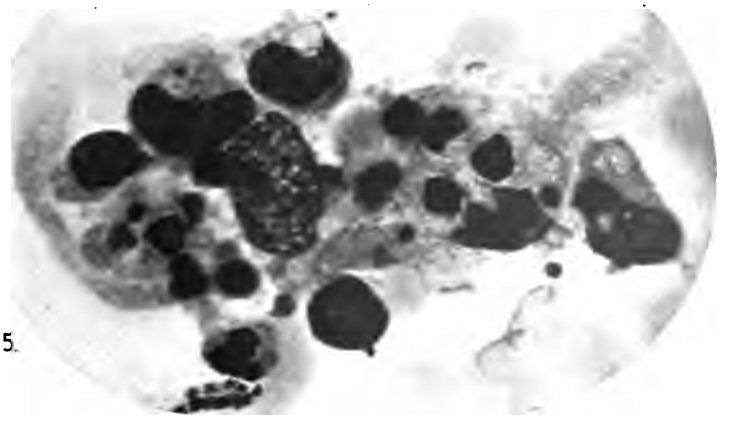
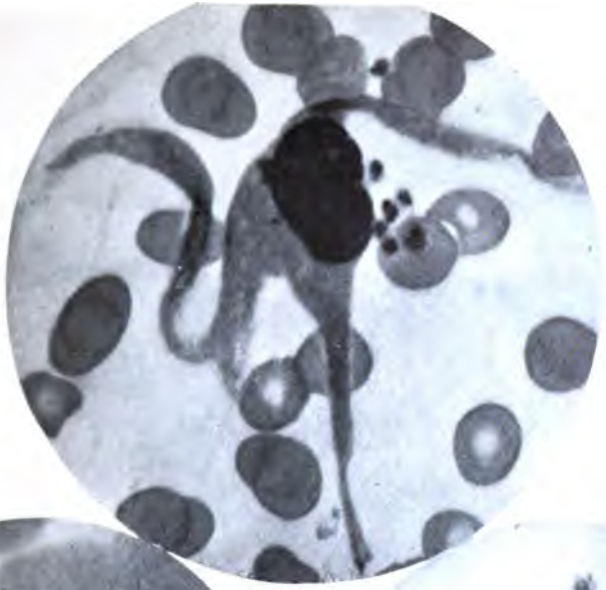


Fig. 5.



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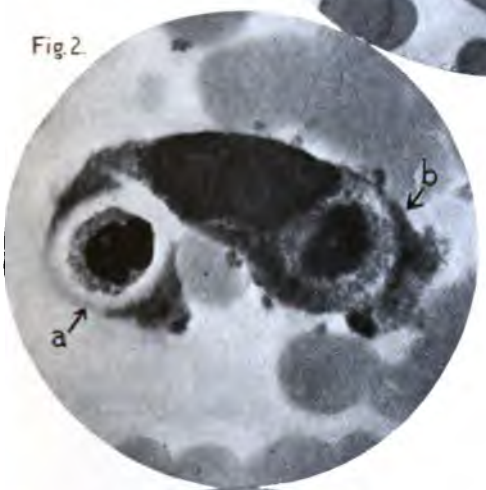

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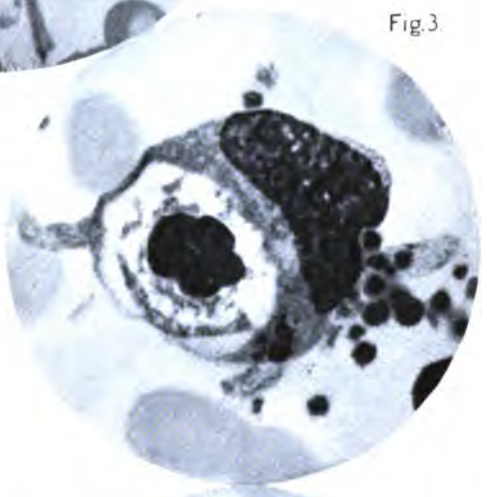

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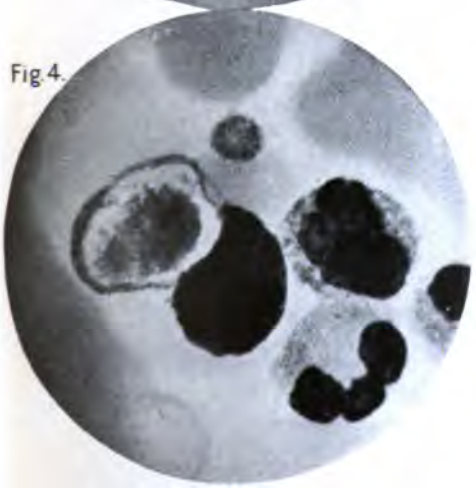

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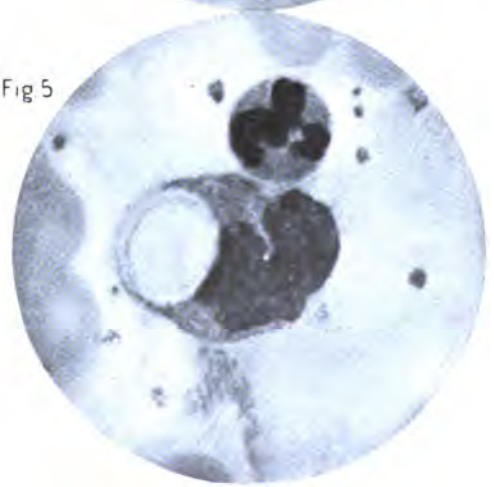

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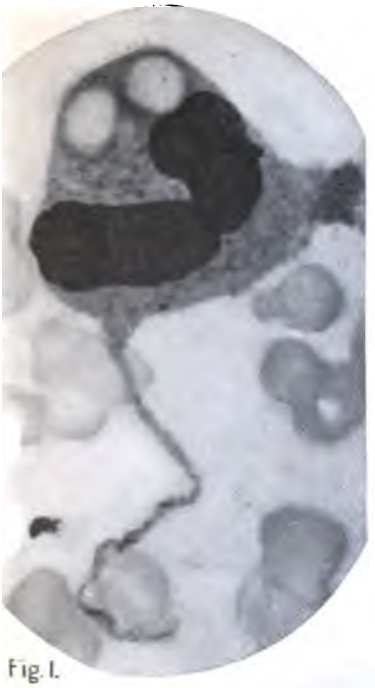

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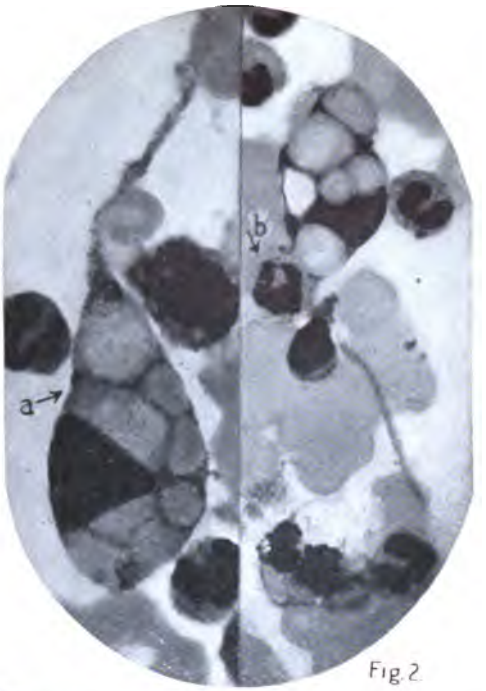

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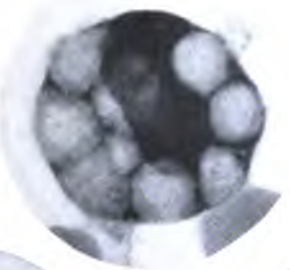

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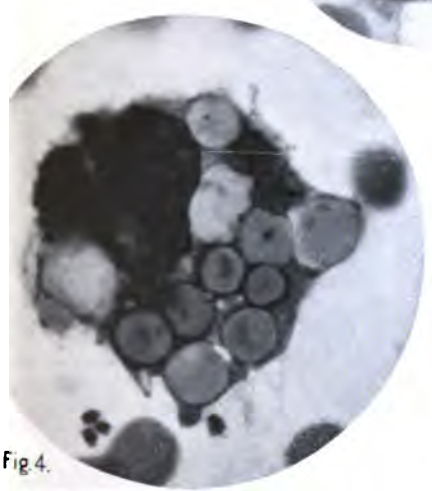

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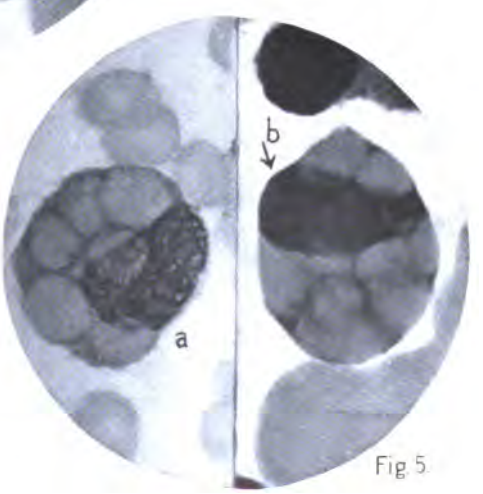

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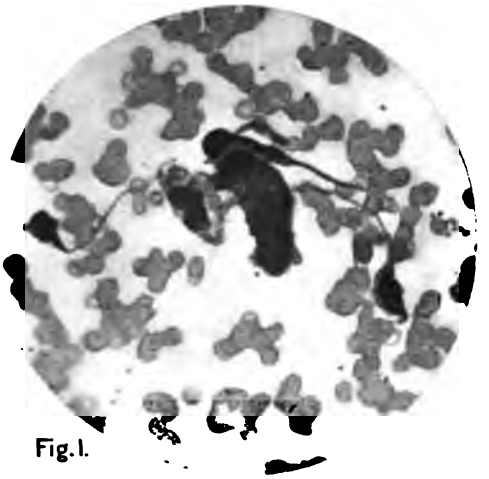

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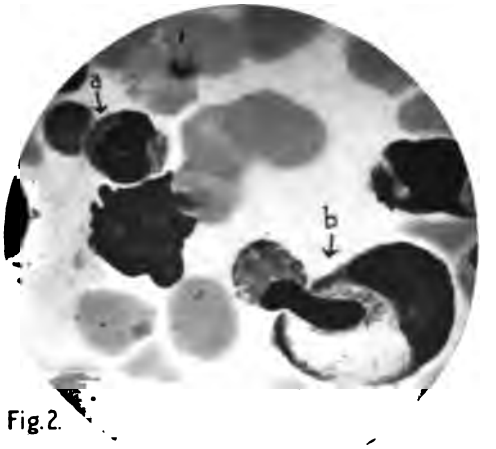

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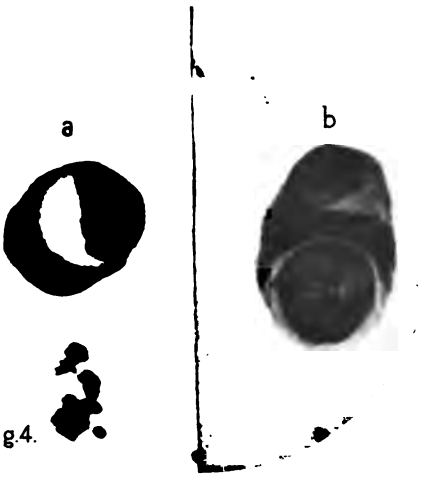

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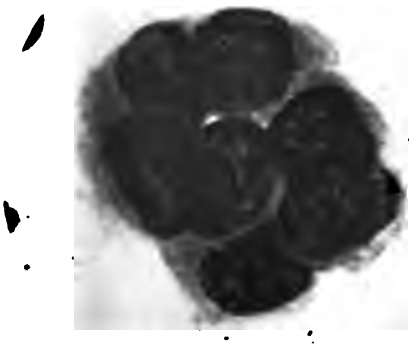

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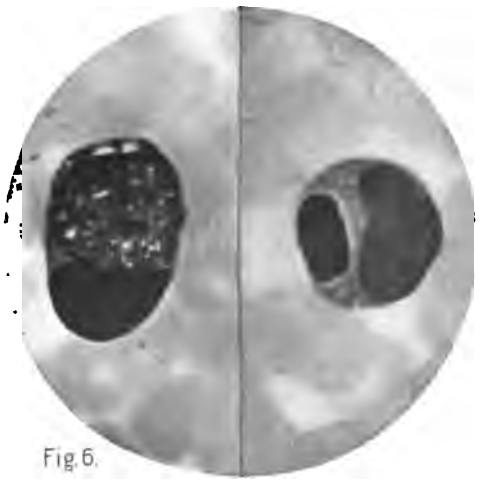


Fig. 6.

FIG. 4. × 1125. A cell with at least eleven red corpuscles included—some of them partially destroyed.

FIG. 5. (*a* and *b*) × 1530. Two cells full of inclusions. In *b* twelve red corpuscles can be counted at different levels.

PLATE X.

FIG. 1. × 450. Two lymphocytes with thin processes widened at the ends. Each of the ends contains a piece of the cell-nucleus.

FIG. 2. × 1125. (*a*) Polynuclear cell with threads from the nucleus around an inclusion. (*b*) Lymphocyte with polynuclear cell partially ingested.

FIG. 3. × 1530. A lymphocyte which has ingested another lymphocyte which itself contains inclusions.

FIG. 4. (*a* and *b*) × 1700. Two small lymphocytes with inclusions.

FIG. 5. × 1530. A giant cell taken from a smear from the patient's spleen.

FIG. 6. × 1530. Leucocytes (lymphocytes) with inclusions; from the blood of the guinea-pig after the patient's blood had been injected into the subcutaneous tissue of the animal.

TRANSPLANTATION IN MASS OF THE KIDNEYS.[1]

By ALEXIS CARREL.

(From the Rockefeller Institute for Medical Research, New York.)

I. DEFINITION.

This operation consists of extirpating from a first animal both kidneys, their vessels and the corresponding segments of the aorta and vena cava, their nerves and nervous ganglia, their ureters and the corresponding part of the bladder (Plate XI); of placing this anatomical specimen into the abdominal cavity of a second animal whose normal kidneys have been previously resected and the aorta and vena cava cut transversely (Plate XII); and of suturing the vascular segments between the ends of the aorta and vena cava, and of grafting the flap of bladder onto the bladder of the host (Plate XIII).

II. INTRODUCTION.

The purpose of the transplantation in mass of the kidneys is to reconstruct, as safely and perfectly as possible, the urinary system, when it has been suppressed by a double nephrectomy, and to study the functions of the new kidneys. It would be important to know whether kidneys extirpated from an animal and transplanted on another animal after a suspension of life of some duration, can resume efficiently their functions. No therapeutic value can be expected from the graft of kidneys unless the secretion of the new organs should be practically normal. In order to ascertain whether or not transplanted kidneys functionate in a normal way, the crucial test is certainly the grafting on one animal, having undergone previously a double nephrectomy, of both kidneys extirpated from another animal. The efficiency of the transplanted organs would be absolutely demonstrated if the host lived in good health and secreted normal urine.

[1] Received for publication October 30, 1907.

The physiology of transplanted kidneys is not known, the main purpose of the experimenters having been until now to reëstablish the circulation through the organ, and not to study its functions. When gangrene did not occur and the kidney secreted a little urine, the operation was considered a success. As the normal kidneys of the host were not resected, it was not possible to appreciate the degree of usefulness of the transplanted organ. All these experiments were made by a method that may be called simple transplantation. It consists of dissecting the kidney and cutting its vessels, extirpating and transplanting it onto some other part of the body of the same or another animal and suturing the renal vessels to other vessels, such as the jugular vein and common carotid artery, or iliac vessels, or even renal vessels.

In 1902 the first attempt of transplanting a kidney was made by Ullmann.[2] He removed a dog's kidney and transplanted it into the neck, the renal artery being united to the carotid artery and the renal vein to the external jugular vein by means of Payr's protheses. The end of the ureter was sutured to the skin and fluid flowed from the ureter, but no analysis of it seems to have been made. Three months after this communication Ullmann[3] reported having transplanted the kidney of one dog into another, and the kidney of a dog into a goat. On macroscopical examination of the kidney transplanted into the neck several necrotic areas were seen. No analysis of the urine was published.

The same year, I performed[4] on dogs several transplantations of the kidneys, at the University of Lyons. The carotid and jugular of a dog were dissected and prepared for anastomosis. The right kidney, having been extirpated, with its vessels and its ureter, was put into the cervical wound. End to end anastomoses by continuous sutures of the renal artery to the carotid, and of the renal vein to the jugular, were performed. The end of the ureter was united to a small opening of the skin a little above the sternum. After reëstablishment of the circulation a clear fluid began flowing from the ureter. Septic complications occurred in every case, and permanent results were not observed. No analysis of the urine was made. In 1902 Decastello[5] also reported experiments on the transplantation of the kidneys. He extirpated the kidney of a large dog and transplanted a kidney from another dog into its place, uniting the vessels by means of protheses. The animal lived forty hours, during which time 1,200 cubic centimeters of urine, containing a great deal of albumin and many casts, were secreted. In 1903 Carl Beck[6] of Chicago performed a transplantation of the kidney by using Murphy's method of anastomosing blood-vessels.

[2] Ullmann, *Wien. klin. Woch.*, 1902, xv, 281.
[3] Ullmann, *ibid.*, 707.
[4] Carrel, *Lyon méd.*, 1902, xcviii, 859.
[5] v. Decastello, *Wien. klin. Woch.*, 1902, xv, 317.
[6] Beck, verbal communication.

In 1905 Floresco[7] performed the transplantation of the kidney into the cervical and the inguinal regions, and in every case gangrene occurred. Then he grafted the kidney in the lumbar region; a kidney was extirpated from a dog and transplanted on another dog which had undergone the resection of one kidney, the renal vessels being united to the renal vessels of the host by continuous suturing and the ureter to the skin or to the ureter of the host. These experiments gave few facts about the functions of the kidneys. In one case a sample of fluid flowing from the ureter five days after the operation was examined and presented the characters of urea. But on the tenth day the organ was found necrosed. Floresco transplanted the kidney five times into the lumbar region. In three cases necroses occurred. In two cases the animal is reported to have lived in good health. Definitive results were not published. No analysis of urine secreted by the transplanted kidney was made.

In 1905 Guthrie and I[8] examined the functions of a transplanted kidney. The operation was performed in the Physiological Department of the University of Chicago. The kidney of a small dog was transplanted into the neck, the renal artery being sutured to the carotid artery, the renal vein to the external jugular vein and the ureter to the œsophagus. When examined three days later, the kidney was somewhat larger and its hue darker than normal. The pulsations of the renal artery were strong. The secretion of urine by the transplanted kidney was about five times more rapid than by the normal one. The intravenous injection of normal saline solution caused no change in the rate of secretion in the normal kidney, but markedly increased the rate of secretion in the transplanted organ. The constituents of the urines were similar, but the chlorides appeared more abundant in the urine from the transplanted kidney, while the organic sulphate, pigments and urea were more abundant in the urine from the normal organ.

This experiment showed that the secretion of a transplanted kidney need not be very different from the normal. However, the kidney was in an unfavorable condition. The denervation alone could not be responsible for the exaggeration of the circulation of the organ. In the neck or in the inguinal region the blood pressure is different from the pressure to which the kidney is used. Besides, its vessels, the renal vein especially, are exposed to many causes of compression and disturbance. Therefore, we gave up the idea of transplanting the kidney in a region other than the lumbar region, as Floresco did.

In 1907 Stich[9] published the results of a transplantation of the kidney in the lower abdominal region. This experiment was performed at the surgical clinic of Garre, in Breslau. The kidney was transplanted into the iliac region, the renal vessels being anastomosed, end to end, to the iliac vessels, and the end of the ureter being grafted into the bladder. The normal kidneys of the animal were not removed, and consequently, the usefulness of the transplanted organ could not be determined. The circulation remained excellent and the macroscopical and microscopical examinations showed that the organ was practically normal. The iliac region is certainly much better than the cervical region, but

[7] Floresco, *Jour. de Physiol. et de Path. generale*, 1905, vii, 27, 47.

[8] Carrel and Guthrie, *Science*, 1905, xxii, 473; *Comp. rend. de la Societé de biologie*, 1905, ii, 669.

[9] Stich, *Archiv f. klin. Chirurgie*, 1907, lxxxiii, 494.

it is probable that perfect functional results can be obtained more easily by putting the kidneys in their normal location in the lumbar region.

In 1906 I made with Guthrie[10] a few transplantations of the kidney into the lumbar region. The renal artery and vein were implanted on the wall of the aorta and vena cava by the patching method. By this method occurrence of gangrene is almost impossible, for the wall of the renal vessels is respected and the suture is made between the edges of the arterial and venous patches and the openings of the aorta and vena cava of the host. The circulation was excellent. Nevertheless there were almost always some small changes in the direction, or the situation, and in the length of the vessels, or some twisting of the vein around the artery, or a little sclerosis of the connective tissue which produced a slight obstruction of the venous circulation, chronic congestion, and after a few weeks, marked lesions of the kidney. Such an organ is not proper for the study of the functions of a transplanted kidney, for its secretion is abnormal. Its lesions and troubles of function are not due to the fact of its having been transplanted, but merely of its having been transplanted with an imperfect technique.

For studying the functions of transplanted kidneys, the transplantation in mass appeared to be the ideal method. It permits an almost perfect reconstruction of the urinary apparatus, and prevents the occurrence of gangrene and, in a large measure, of secondary lesions of the parenchyma, since the renal vessels themselves are not disturbed and the ureters not severed. It may preserve a part of the nervous system of the organs, and places the organs in a condition as near as possible to normal.

The first transplantation in mass of the kidneys was performed in 1906 by Guthrie[11] and myself in the Physiological Laboratory of the University of Chicago.

Both kidneys and the upper part of the ureters of a dog, together with their vessels, nerves, nervous ganglia, the surrounding connective tissue, the suprarenal glands, the peritoneum and the corresponding segments of the aorta and vena cava were removed. The mass was then placed in a vessel of isotonic sodium chloride solution. The aorta and vena cava of a bitch were cut a little

[10] Carrel and Guthrie, *Jour. of Amer. Med. Assoc.*, 1906, xlvii, 1648.

[11] Carrel and Guthrie, *Science*, 1906, xxiii, 394; *Comp. rend. de la Societé de biologie*, 1906, i, 465.

above the mouth of the ovarian vessels. The kidneys of the first animal were then removed from the salt solution and put into the abdominal cavity of the second, and the segments of the aorta and vena cava were interposed, by biterminal transplantation, between the cut ends of the aorta and vena cava. The circulation was reestablished, after having been interrupted for one hour and a half. The kidneys immediately became red and turgid, as after a simple transplantation, but about half an hour later the state of the circulation became normal. Clear urine flowed abundantly from the transplanted ureters, which were united to the normal ones.

Both normal kidneys were then removed. Two hours after the operation the animal walked about her cage. In the afternoon she drank and urinated copiously. The following day and subsequently, up to the eighth day, her diet consisted largely of meat, which she took hungrily. In general her condition was normal. During this period the urine remained clear, showing no evidence of blood. The total amount appeared to be somewhat increased. In the seventh and eighth days several samples were collected and analyzed, the results of which showed a slight variation in composition, but entirely within normal limits. The only abnormal constituent detected was coagulable proteid, the largest amount present in any of the samples being less than 0.25 per cent.

On the ninth day she vomited. A diagnosis of stenosis of the bowels by adhesions was made. On the tenth day the urine was analyzed and its composition was practically the same as before. As the animal was vomiting almost continuously, she was etherized and a laparotomy performed. We found localized peritonitis on the right side of the abdomen, with kinking of several loops of intestines, the mass being very strongly bound down by adhesions. The circulation of both kidneys was found to be perfect. There was an enormous hydronephrosis on one side.

Afterwards a few other transplantations on dogs and cats were performed without obtaining better results. The animals died rapidly from intestinal, peritoneal or ureteral complications.

In 1907 at the Rockefeller Institute, I modified and improved the technique in order to suppress, as much as possible, the occurrence of complications. A few operations were made on dogs and on cats and it appeared soon that, in spite of the difficulties due to the smallness of the vessels, cats are better adapted from an anatomical standpoint for this operation than dogs. When the details of the operation had been worked out fourteen experiments were performed from February to October, 1907. Progressively the technique was improved and the cause of the complications which occurred ascertained, and as far as possible suppressed. The actual technique, however, must not be considered as a definitive one. It will be modified in many respects, as greater experience in performing it is obtained.

III. TECHNIQUE.

The transplantation in mass of the kidneys can be described as consisting of four stages: (*a*) preparation of the kidneys; (*b*) perfusion and extirpation of the kidneys; (*c*) preparation of the host; and (*d*) graft of the kidneys into the abdomen of the host.

It is evident that all resources of modern surgery must be used to prevent infection and shock after such an operation, which necessitates a large transverse incision of the abdominal wall, the evisceration of the intestines and the spleen, a double nephrectomy, the stopping of the aortic and caval circulations, the section of the aorta and vena cava, and the opening of the bladder. The animal is handled and operated on with the same rigid asepsis and care used for a human patient in a well-equipped hospital.

The order of the succession of the different stages of the operation is variable. We shall describe the simplest form, when the four stages are united in two: preparation and extirpation of the kidneys; preparation of the host and graft of the kidneys.

A. *Preparation and Extirpation of the Kidneys.*

A first animal is etherized. After shaving and sterilization of the abdominal and lumbar regions, the antero-lateral wall is cut transversely at the level of the umbilicus to about one half or three quarters of the circumference of the animal. The intestines are eviscerated and put on the left side of the body. Thus both kidneys and the lumbar region are largely exposed.

1. *Dissection of the Kidneys.*—The peritoneum is cut all around the kidneys in a rectangular shape, the short sides being along the external edge of the kidneys, the long sides uniting the ends of the organs and being perpendicular to the vena cava. Care must be taken not to wound the ureters and the vena cava. Through the peritoneum it is easy to see the point of implantation of the renal veins. At about one centimeter above the mouth of the right renal vein the vena cava is dissected and isolated from the lower end of the right suprarenal gland. On the left side of the vessel, just above the left renal vein, is often the mouth of the left suprarenal vein. This vein is ligated and cut, if the left suprarenal gland is not to be transplanted with the kidneys. Afterwards, the vena

cava is dissected on a point located at two or three centimeters below the mouth of the left renal vein. The left genital vein is ligated and cut. Then the aorta is dissected at two points located one and one half centimeters above and below the renal arteries. The kidneys are detached from the lumbar region by cutting the loose connective tissue which is interposed between them and the muscular plane. The posterior part of the aorta and vena cava is dissected. The posterior collateral branches of these vessels are ligated and cut.

The ureters are dissected from the lower edge of the peritoneal flap to the bladder. Their vessels must be carefully respected. Afterwards the part of the bladder on which the ureters are implanted is dissected. The musculo-peritoneal layer of the bladder is cut by a circular incision located at about one centimeter from the mouth of the ureters. Then the mucous membrane is easily seen and cut, in such a way, with the scissors, that the mucous flap is larger than the muscular one. The ureters and the flap of bladder are still fixed by the rectum and the uterus. The uterus is cut, and the meso-colon severed as far as the cæcum. The small intestine is cut and through the solution of continuity the ureters and the fragment of bladder are removed. Great care is taken to prevent infection by the section of the intestines.

If the left suprarenal must be transplanted, its upper vessels are ligated and cut. The last connective tissue adhesions are severed. The anatomical specimen is yet adherent to the animal by the aorta and vena cava.

2. *Stopping of the Circulation, Perfusion and Extirpation of the Kidneys.*—The aorta is clamped below the diaphragm. A glass cannula is introduced through the lower part of the abdominal aorta and connected with an irrigator containing Locke's solution at the temperature of the laboratory, which was from 26° to 37° C. The vena cava is cut one centimeter above and two or three centimeters below the renal veins. Then the aorta is washed and the kidneys are perfused with Locke's solution. In the first experiments they were washed until their surface became yellowish white and the fluid flowing from the vena cava was perfectly clear. In the last experiments the perfusion was much less complete. It was stopped

as soon as the blood in the vena cava appeared to be greatly diluted, and when there was still a good deal of blood in the kidneys.

Next the aorta is cut one centimeter or one and a half centimeters above and about two centimeters below the renal arteries. The anatomical specimen is removed (Plate XI) and put into a jar of Locke's solution at the temperature of the laboratory.

B. *Preparation of the Host, Double Nephrectomy and Graft of the New Kidneys.*

A second animal of the same size or a little larger than the first one is etherized. The abdomen and the lumbar region are clipped, shaved and carefully sterilized. The abdominal wall is transversely cut at the level of the umbilicus from one lumbar region to the other. The intestine and the spleen are eviscerated and wrapped in a towel or in a greased silk pad, and put outside on the left side of the body. They are protected there by a small wool blanket, in order to prevent cold and the consequent shock. The aortico-renal region is then largely exposed. When the bladder is too distended by urine, it is put outside of the abdominal cavity and covered with greased pads.

1. *Extirpation of the Kidneys* (Plate XII).—The lumbar peritoneum is cut longitudinally on the middle line, its edges dissected at the level of the renal veins. The pedicles of the kidneys are dissected and a ligature is put on each structure, artery, vein and ureter. Afterwards through the longitudinal peritoneal incision, both kidneys are extirpated in such a manner that the constitution of the renal region is not disturbed at all. Both suprarenal glands are respected, their lower vessels being sometimes ligated. The left genital vein is ligated.

2. *Preparation of the Vessels.*—The vena cava and the aorta are prepared for anastomosis. The vena cava is dissected at the point of implantation of the renal and the left suprarenal veins. One or two lumbar collaterals, and sometimes the right genital vein are ligated. The temporary hæmostasis is secured by *serre fines* especially modified for this purpose. A *serre fine* is put on the vena cava four centimeters below the renal veins, and another one as high as possible, about two centimeters above the right renal

vein. Then the region on which the renal veins are implanted is resected (Plate XII). Through the cut ends of the vessels a curved glass cannula is introduced, and the blood is washed out with Locke's solution. A little vaseline is put into the lumen and on the external part of the vein. The aorta is generally dissected just below the renal arteries; the first two lumbar collaterals are ligated, a *serre fine* is put on the wall of the vessel just below the renal arteries, and another three centimeters lower. It is better, but involves greater risk, to dissect the aorta above the renal arteries, in order to place the *serre fine* higher and to make the anastomosis a few millimeters below the implantation of these vessels. The relations of the thoracic duct and of the aorta are so close at that point that the duct is exposed to injury while the aorta is being dissected. It is much safer to put the higher *serre fine* below the renal arteries. Generally the aorta is cut two centimeters below the renals, or sometimes a very short segment, with the implantation of the first two lumbar collaterals, is resected. The aorta being very elastic there is immediately a gap of two or three centimeters between the cut ends of the vessel (Plate XII). The external sheath is resected for a short distance. The blood is washed out from the lumen of the vessel with Locke's solution. The lumbar region is carefully washed with Locke's solution and dried with gauze in order to remove all traces of blood. Then the vessels are washed again, and covered with a thin layer of vaseline. Vaseline is also put on the surounding anatomical structures.

3. *Transplantation of the Kidneys.*—The anatomical specimen is removed from its jar and put into the abdominal cavity. Each kidney is introduced through the incision of the lumbar peritoneum into the corresponding renal region. The new kidneys, being about the same size as the extirpated ones, fill exactly their place. The aortic and caval segments are interposed between the cut ends of the aorta and vena cava. The ends of the vascular segments are washed, if necessary, and greased with vaseline. Afterwards the small region of the vessels is circumscribed with black silk towels, for it is necessary to protect the threads from all contact with blood or with plasmase of the tissues.

4. *Anastomoses of the Vessels and Reëstablishment of the Circu-*

lation.—The ends of the aorta are united to the ends of the aortic segment by continuous suturing with straight needles, No. 16, and silk thread boiled in vaseline. Care is taken to express the vaseline from the lumen of the vessel before completing the suture. Afterwards the anastomoses of the ends of the venous segments to the ends of the vena cava are made. The wall of the vein is very thin, and it is necessary to apply intima to intima while suturing, that is, to make a slight eversion of the wall. It is the safest way to prevent inclusion of the external sheath into the line of sutures.

After completion of the anastomoses dry gauze is applied on the anastomoses and the *serre fines* are removed first from the vena cava and secondly from the aorta. For about two minutes a slight compression is made in the region of the anastomosis. Afterwards the gauze is removed and, if there is still a little leakage, one or two complementary stitches are added. It is necessary that no blood at all flow from the line of sutures. The hæmostasis must be absolute.

As soon as the circulation is reëstablished through the aorta, the kidneys begin to be injected with blood, and generally after a few minutes, they assume their normal appearance. The duration of the interruption of the circulation is about one hour. Gauze with Locke's solution is put on the vessels.

5. *Graft of the Flap of Bladder on the Bladder of the Host, and End of the Operation.*—The ureters are extended on the free surface of the peritoneum along the right side of the rectum and along the right uterine horn. The bladder is isolated by pads from the peritoneal cavity. On the middle line of its posterior face a longitudinal incision is made. The flap of bladder is grafted into that opening by muco-mucous, and musculo-muscular continuous sutures. Then the bladder is put back into the abdominal cavity.

The arterial and venous anastomoses are examined again, and if there is slight leakage, it is stopped. If a little blood flows from some small vessels of the connective tissue a ligature is applied, even if the hæmorrhage is exceedingly small. The vaseline and the blood which may be present in the connective tissue around the vessels are washed out with warm Locke's solution.

The lumbar region is closed by suture of the longitudinal peritoneal incision. Thus both kidneys are fixed in their normal location. The intestines and the spleen are put back into the peritoneal cavity. If the intestines are a little cold about one hundred grams of warm Locke's solution are injected into the peritoneal cavity.

The peritoneum and the abdominal muscles are closed by two planes of continuous silk sutures. The skin is sutured with catgut. The dressing consists of talcum powder, gauze, cotton, bandage and a linen shirt.

After the operation the animal is put for two hours into a cage heated to about 30° C. and then into an ordinary cage with screen floor or saw dust. No special care is taken. The animal is given meat and milk one day after the operation. The dressing is removed after about six days. When the urine is to be examined, the animal is put into a metabolism cage with fine screen floor. Only incomplete analyses of urine have been made by using the ordinary clinical methods. In spite of their lack of accuracy they are sufficient to prove that the kidneys functionate. It is not intended, in this article, to analyse minutely the changes in the secretion of transplanted kidney, but merely to show that after transplantation they can resume their function efficiently. This is demonstrated as much by the general condition of the animal as by the physical and chemical characters of the urine secreted by the new kidneys.

IV. EXPERIMENTS.

Fourteen experiments have been performed.[12] Two animals whose ureteral anastomoses were defective died soon after the operation. No excretion of urine was observed from the bladder, because it flowed into the retroperitoneal spaces. Three other animals, operated on under unfavorable condition died almost immediately of shock. These five experiments will not be reported. Thus nine cases only will be described.

Experiment 1. February 25, 1907.

Extirpation of the Kidneys.—Middle-sized male cat. Etherization and semi-

[12] Several of these operations were made with the aid of Mr. R. D. McClure of Johns Hopkins University, whom I wish to thank for his assistance.

circular transversal laparotomy. Evisceration. Dissection and isolation from the surrounding structures of both kidneys, the left suprarenal gland, their vessels and the corresponding segments of the aorta and vena cava, their nervous system, and the upper part of the ureters, which are cut about 6 centimeters below the hilus. Opening of the lower part of the abdominal aorta. Animal killed by hæmorrhage and opening of the diaphragm. Section of the vena cava 1.5 centimeters above and 3 centimeters below the mouth of the renal veins. Introduction of a cannula through the abdominal aorta. Washing of the aorta and perfusion of the kidneys with very hot (by accident) Locke's solution, until their color became yellowish-white and the fluid flowed perfectly clear from the vena cava. Then section of the aorta about 1.5 centimeters above and 1.5 centimeters below the mouth of the renal arteries. Anatomical specimen removed and put into Locke's solution at the temperature of the laboratory (30° C.).

Transplantation of the Kidneys.—Male, young, black cat, good health. Etherization. Semi-circular transversal laparotomy. Evisceration of the intestine on the left side of the body. Longitudinal incision of the lumbar peritoneum in the middle line, through which both kidneys are dissected and extirpated. Dissection of the vena cava at the level of the renal veins. Ligation of two posterior collateral branches. Dissection of the aorta, which is isolated from the thoracic duct. Ligation of both first lumbar collateral branches. Temporary hæmostasis of a segment of aorta by two *serre fines* put on the vessel just above and 4 centimeters below the renal arteries. Section of the aorta a little above the mouth of the first lumbar branches. Resection of a segment of aorta about 5 millimeters long. Temporary hæmostasis of the vena cava by two *serre fines* placed just above the right renal vein and the right spermatic vein. Resection of the point of implantation of the renal veins. Washing of the ends of the vessels with Locke's solution and greasing with vaseline.

The anatomical specimen is then removed from the jar and put into the abdominal cavity, each kidney being placed under the lumbar peritoneum in its normal location. Interposition of the aortic and caval segments between the cut ends of the aorta and vena cava. Anastomosis of the vessels by suture. No expression of the vaseline from the vessels before completion of the suture. The *serre fines* are taken out and the blood flows through the aorta and vena cava. Slight leakage from the venous anastomoses, which stops by compression. No leakage of the arterial anastomoses.

Very slow reëstablishment of the circulation through the kidneys. Nevertheless, after a few minutes, the right kidney assumes a rosy color, without blue or white spots, while the left kidney remains paler. The lower end of the left kidney is white. Small incision made at this point. Slight hæmorrhage of red blood mixed with vaseline. Progressive improvement of the circulation of the left kidney. After thirty minutes its color is rosy. Circulation almost normal.

Invagination of the end of the transplanted ureters into the upper end of the ureters of the host, and fixation of the invagination by three stitches. Closing of the lumbar peritoneal incision. Reintegration of the intestines into the abdominal cavity. Closing of the abdominal wall by three planes of catgut sutures. Gauze and cotton dressing, linen shirt.

February 25, 4 p. m. Cat in good condition. A little depressed.

February 26. Cat in good condition, walks about its cage, drinks water, but does not eat. Femoral pulse normal. A little urine.

February 27. Cat in good condition, drinks water and milk, walks, does not eat meat.—9 a. m. Urinates abundantly.—4 p. m. Urinates again. Yellowish urine, acid reaction, albumin present.

February 28. Good condition. Normal femoral pulse. Animal drinks milk, urinates abundantly, walks about the room. Clear, pale, yellow, acid urine. Albumin.

March 1. Animal in very good condition. Urinates abundantly. Clear, yellowish urine. Less albumin.

March 2. Same condition.

March 3. Animal ill, does not drink milk. Coughs, discharge through the nose. Urinates.

March 4. Animal very ill, coughs, diarrhœa, discharge through the nose. Urinates abundantly.

March 5. Same condition. Animal emaciated. Abundant urine. Drinks water.

March 6. Animal in better condition. Diminution of the discharge through the nose and of the diarrhœa. Drinks milk. Walks about its cage and the room.

March 7. Much better condition. No more discharge. A little diarrhœa. Less abundant urine. Animal hungry and drinks milk, eats fish and raw meat.

March 8. Animal drinks milk and eats raw meat. Urinates. Walks about the room.

March 9. Animal ill, refuses to eat. Urinates as usual, stays in cage.

March 10. Animal very ill. Not able to jump from its cage to the floor. No vomiting, no dyspnœa, no shaking. Urinates. Pressure on the abdominal wall is painful. In one point dressing is wet.—11 a. m. Etherization. Section of the dressing. Several loops of intestines in the gauze, the abdominal wound being almost completely disunited: premature resorption of the catgut. The intestinal loops are partially protected by the omentum which is adherent to the lower edge of the abdominal wound. Comparatively little inflammation of the peritoneum. However, four intestinal loops are adherent to the gauze dressing and very much inflamed. Adhesions are detached and intestine washed. Direct examination of the kidneys: normal peritoneal covering, normal location, marked enlargement, almost normal consistency. They look like normal hydronephrotic kidneys. Washing of the abdominal cavity with warm water. Closing of the abdominal wound by silk suture. Shock. Afterwards respiration and pulse become progressively almost normal—4 p. m. Animal able to walk.

March 11. Died in the morning.

Autopsy.—General peritonitis.

Macroscopical Examination.—Both kidneys in normal location, covered with transparent peritoneum. Normal hue, increased size. Normal consistency.

The specimen is not dissected, but preserved as demonstration specimen. Incision on the external edge of the left kidney. Urine under pressure flows from the incision. The surface of section is a little congested. No infarction. Dilatation of the pelvis and calices. It is a typical hydronephrotic kidney. Right kidney not opened, but a knife is introduced deeply into the renal substance, and urine escapes along the blade. Same hydronephrotic condition on the right side. Opening of the vena cava. Anastomoses perfect. No dissection of the aorta. No dissection of the ureters. Specimen preserved in formalin.

Microscopical Examination.—Fragment of the left kidney in Zenker's fluid stained in hematoxylin and eosin. Glomeruli well preserved. In some of them slight exudate between capillaries and the capsule. Secreting tubules slightly dilated. Epithelium in good condition; brush border well defined, nuclei normal. Excretory tubules very much dilated. Around some of them slight small-cell infiltration. A little dilatation of the blood-vessels. Appearance of an ordinary hydronephrotic kidney.

Experiment 2.—March 14, 1907.

Extirpation of the Kidneys.—Small pregnant cat etherized and killed by aortic hæmorrhage. Dissection and isolation in one mass of the kidneys, their vessels, their nerves and the upper part of the ureters. Left suprarenal gland not extirpated. By an intra-aortic injection of Locke's solution both kidneys are washed out very thoroughly, until all blood is expelled and their color becomes pure yellowish-white. Anatomical specimen extirpated and put in Locke's solution at the temperature of the laboratory (30° C.).

Transplantation of the Kidneys.—Large, gray, young, male cat. Etherization. Semi-circular transversal laparotomy. Evisceration. Extirpation of both normal kidneys. Dissection of the aorta and vena cava. Ligature of the lower suprarenal vein and section. Ligation of the first lumbar collateral branches of the aorta. Temporary hæmostasis of the aorta by tapes fixed by *serre fines,* and of the vena cava by *serre fines* put directly on the wall of the vessel. Resection of a narrow segment of aorta in which the first lumbar arteries are implanted. Resection of a long segment of vena cava where are implanted the inferior suprarenal vein and both renal veins.

The anatomical specimen is then put into the abdominal cavity, the kidneys being in their normal location and the vascular segments interposed between the cut ends of the aorta and vena cava. Arterial and venous anastomoses by the ordinary method. Reëstablishment of the circulation through the vena cava. Leakage at one point of the lower anastomosis; hæmorrhage controlled by one supplementary stitch.

Reëstablishment of the circulation through the aorta. Hæmorrhage of the upper anastomosis, which is controlled by two additional stitches. Slow reëstablishment of the circulation through the kidneys. Appearance of both kidneys normal twenty minutes after the reëstablishment of the arterial circulation.

Anastomoses of the ureters to the upper portion of the ureters of the host by invagination. Suture of the lumbar peritoneum. Reintegration of the intestines in the abdominal cavity. Closing of the abdominal wound by two planes of silk suture and one plane of catgut suture. Gauze and cotton dressing. Shirt.

March 15. Animal in good condition, drinks milk and eats raw meat. Large amount of clear urine. In the afternoon several fits of abdominal pain.

March 16, 9 a. m. Animal less well, refuses to eat, drinks a great deal of water and milk. Since yesterday at 6 p. m. 130 c.c. of urine. Urinates again at 10.30 a. m. yellow, clear, acid urine. A great many spermatozoa, no red blood corpuscles, a little albumin. In the afternoon, fits of abdominal pain, vomited once, no feces. In the evening animal very ill.

March 17. Animal died in the morning.

Autopsy.—A little reddish fluid in the peritoneal cavity. A loop of small intestine is found dilated and of dark color: volvulus. Both kidneys are normal in size, color and consistency. Arterial and venous anastomoses perfect.

Experiment 3.—June 13, 1907.

Extirpation of the Kidneys.—Middle-sized male cat. 10.38 a. m. Etherization. Kidneys exposed and dissected the same as before. Dissection of the aorta and vena cava at two points about 1.5 centimeters above and 2 centimeters below the mouth of the renal vessels. Section of the ureters about 6 centimeters below the hilus. The animal is killed by chloroform at 11.15 a. m. Section of the vena cava. Perfusion of both kidneys through the aorta with Locke's solution at the temperature of the laboratory (30° C.). Complete washing. The fluid from the vena cava is clear, and the kidneys are yellowish-white. Section of the aorta 1 centimeter above and 2 centimeters below the renal arteries. Then the kidneys, the left suprarenal gland, the upper part of the ureters with their vessels and their nerves are removed and put in Locke's solution.

Transplantation of the Kidneys.—Large, yellow, male cat. Etherization. Semi-circular transversal laparotomy. Evisceration of the intestines and the spleen and extirpation of both kidneys. Ligature of the lower suprarenal vein, of two venous collaterals of the vena cava, and the first pair of aortic lumbar collaterals. Dissection of the aorta and vena cava and temporary hæmostasis with *serre fines*. Transverse section of both vessels. The anatomical specimen is then put in the abdominal cavity, the new kidneys being in the same location as the kidneys of the host. Anastomosis of the aortic and caval segments. Re-establishment of the venous and arterial circulation at 12.20 p. m. No leakage of the anastomoses. Immediate reëstablishment of the circulation through both kidneys. No blue or white spots. Normal color and consistency. No apparent vasodilation.

Anastomosis of the ureters by invagination. Suture of the lumbar peritoneum. The intestines and the spleen are replaced into the peritoneal cavity. A few cubic centimeters of Locke's solution are let into the peritoneum. Closing of the abdominal wound by two planes of silk sutures and one plane of catgut. Gauze and cotton dressing. Shirt. Animal is put in a metabolism cage.

June 13, 4 p. m. Animal in satisfactory condition, lays down, does not walk, drinks water.

June 14, 9 a. m. Animal in good condition, walks about his cage, and drinks milk. In the jar 250 c.c. of a mixture of urine, vomitus and a little milk.—9.30 a. m. Animal urinates, clear, slightly rosy urine, quantity 42 c.c., reaction acid, density 1.021, a great many blood corpuscles, a few spermatozoa, no casts.

June 15, 9 a. m. Animal in good condition, drinks milk. Urine 110 c.c. mixed with feces.—3.45 p. m. Clear, rosy urine, with many blood corpuscles.—4.35 p. m. Animal urinates again. Urine 38 c.c., clear, slightly rosy, acid reaction, density 1.018, urea 2.5 per 100 c.c., albumin less than 0.50 g. per 1000 c.c. Many red blood corpuscles, a few spermatozoa, no casts.

June 16, 9 a. m. Vomits a little milk. In the jar 205 c.c. urine and milk.

June 17. Animal in good condition, walks about the room, drinks water, but refuses milk and meat. Does not vomit. Urine mixed with feces.

June 18. Urine 125 c.c., yellowish, acid, density 1.016, urea 2.8 per 100 c.c., traces of albumin. Animal refuses to eat.

June 19. Animal grows emaciated, drinks a great deal of water. Urine 164 c.c., pale yellowish, clear, density 1.013, very little albumin, urea 2.3 per 100 c.c. urinates again 35 c.c. at 1 p. m.

June 20. During the night the animal has escaped from its cage, and urinated on the floor. He is emaciated and refuses to eat.—10.30 a. m. Urinates again, urine 35 c.c., clear, density 1.013 with traces of albumin.

June 21, 7.30 a. m. Urine 115 c.c., alkaline reaction. Density 1.012, urea 2.6 per 100 c.c., no albumin.

June 22, 7.15 a. m. Urine 167 c.c., density 1.017, no albumin. Animal is much emaciated, and does not eat.

June 23. Urine 110 g. Animal is very weak, nevertheless he can walk about the room. The dressing is removed. Wound completely healed. Animal very much emaciated, kidneys a little enlarged. Does not vomit.—11 a. m. Transfusion of blood by Crile's method through an anastomosis of the carotid artery of another cat to the external jugular vein. Blood is transfused until the second animal dies from hæmorrhage.

The animal is now in better condition. Pulse much stronger. The mucous membrane of the mouth and the skin of the feet are red. A few minutes after the operation clonic convulsions of the jaws and the limbs. Afterwards tonic convulsions. Tetanus-like appearance.—5 p. m. Died.

Autopsy.—Macroscopical Examination.

Opening of the abdomen a few minutes after death. Excellent healing of the abdominal wound. No adhesions of the intestines. In the gastric region long and narrow strand compressing the duodenum just below the pylorus, without, however, producing complete occlusion. Both kidneys are increased in size, their color, their location and their consistency are almost normal. Section of the right kidney: dilatation of the pelvis and calices, cortex and medulla apparently normal, although a little congested. Capsule normal. Upper part of the ureter greatly dilated. Incision of the right ureter: stenosis of the invaginated part; below this point the ureter normal. The left ureter too is very much dilated and sinuous above the point of anastomosis.

Bladder filled with 35 c.c. yellow, clear urine, density 1020, urea 2.9, no albumin.

Vessels of the kidneys normal. Aorta and vena cava dissected and incised. No thrombosis or stenosis, perfect anastomoses. No sclerosis of the perivascular connective tissue.

Microscopical Examination.—Piece of the right kidney fixed in Zenker's fluid, stained in hematoxylin and eosin. Glomeruli normal, secretory tubules slightly dilated, epithelial cells in good condition. Excretory tubules somewhat dilated. Very few light casts. No interstitial infiltration. At a few points a few mononuclear leucocytes around the tubules.

Experiment 4.—June 19, 1907.

Preparation of the Host.—Young, white and black male cat in very good health. Etherization. Semi-circular transversal laparotomy. Evisceration, dissection and extirpation of both normal kidneys, ligation of the lower suprarenal vein, of two collaterals of the vena cava and of the first lumbar pair. Temporary hæmostasis. Section of the aorta about 2 centimeters below the renal arteries. Resection of the venous segment on which are implanted the renal veins. Washing and greasing of the vascular ends. Then gauze compresses with Locke's solution are put on the operative field and protected with a wool blanket.

Extirpation of the Kidneys.—Middle-aged, pregnant cat. Etherization. Opening of the abdomen by the ordinary method. Veins extremely dilated. When the anatomical specimen, both kidneys, and left suprarenal gland are almost completely isolated, the ureters are dissected as far as the bladder. Resection of a flap of the vesical wall around their mouths. Then section of the uterus, mesocolon and the small intestine near the cæcum. The ureters and the flap of the bladder are removed from the lower part of the abdomen and placed temporarily below the left kidney. Perfusion of both kidneys with Locke's solution at the temperature of the laboratory (37° C.). The anatomical specimen is removed and placed immediately in the abdomen of the host.

Transplantation of the Kidneys.—Each kidney is placed under the peritoneum in its normal location. Anastomosis of the vessels and reëstablishment of the circulation. No leakage. It is noticed that the lower end of the venous segment has been twisted and that it produces a very marked stenosis just above the lower anastomosis. It does not interfere with the circulation of the kidney, the color of which almost immediately becomes normal. The circulation has been interrupted for 42 minutes only. Immediate secretion of clear fluid.

The ureters are placed in the abdominal cavity on the right side of the rectum. Longitudinal opening of the bladder on its posterior face and on the middle line, and graft of the transplanted flap of bladder by muco-mucous and musculo-muscular sutures. Closing of the lumbar peritoneum. Through the lower part of this line of suture a small opening is reserved to allow the ureters to pass from the retro-peritoneal space into the peritoneal cavity. Both ureters are twisted around each other, but, as there is no tension, it probably does not interfere with the flow of urine.

The intestines are put back into the abdominal cavity. Suture of the abdominal wall. Dressing. Shirt.

June 20. Animal in good condition. Urine a little bloody. Does not eat.

June 21. Animal in excellent condition, urinates abundantly, eats a great deal of raw meat.

June 22. Animal apparently in very good health, climbs on roof.

June 23. Dressing is removed, wound completely healed. Animal eats meat and drinks a great deal of milk, urinates abundantly.

June 24. Animal looks well, but refuses to eat.

June 25. Animal in good condition, does not eat. Does not vomit. No feces. Clear, yellow urine, urea 2.2 per cent. A little albumin, less than 0.25 g. per 1000 c.c.

June 26. Animal in good condition, walks, climbs on a wall more than six feet high, but refuses to eat and to drink milk. Then a direct examination of the abdominal cavity is decided upon.

10 a. m. Etherization. Longitudinal laparotomy on the middle line. No intestinal adhesions to the abdominal wall. A few adhesions between the duodenum, the anterior face of the right kidney and the inferior face of the liver. They are loose and easily detached. The kidneys are covered by normal peritoneum and apparently in excellent condition. Size, color and consistency normal. The surface of both kidneys is rosy, there is no apparent vasodilatation. No sclerosis of the connective tissue between the kidneys. The renal veins and the transplanted segment of vena cava appear to be absolutely normal. Normal pulsations of the aorta and the renal arteries.

Then closing of the abdominal wound by four planes of sutures.

One hour after the operation, the animal walks and seems in good condition.

June 27, 8 a. m. Animal found dead in its cage. Body is still warm.

Autopsy.—9.15 a. m.

Macroscopical Examination.—Peritoneum normal, no fluid, no adhesions. Bladder filled with urine.

Both kidneys are in their normal location, covered with sound peritoneum, and normal in color, size, consistency and connections with surrounding structures. From a point corresponding to the lower end of the incision of the lumbar peritoneum, both ureters adherent to one another, penetrate into the peritoneal cavity and go down toward the bladder along the right side of the rectum. Excellent union of the transplanted flap of bladder to the bladder of the host (Plate XIII).

Incision of the peritoneum and dissection of the kidneys. Their appearance is normal. They are surrounded by their covering of connective tissue, which does not present any sclerosis. Dissection and incision of the aorta and vena cava. Anastomoses healed without any deposit of fibrin. Vena cava is obliterated 1 centimeter above the lower anastomosis at the point of the twisting of the transplanted venous segment. This point is much below the mouth of the renal veins and the obliteration did not interfere with the venous circulation of the kidneys. Both kidneys are opened. Capsules normal. No hydronephrosis. In section they have the appearance of normal kidneys. A piece of the right kidney is fixed in Zenker's fluid.

Lungs, heart, liver, spleen apparently normal. No examination of the brain and medulla.

Microscopical Examination.—Glomeruli normal. No exudate in the glomerular space. Epithelial cells of the tubules generally well-preserved, brush border very apparent, regularly disposed and adherent to the basement membrane. In some places vacuolization of the protoplasm around the nucleus. Exudate in the lumen of the tubules. Epithelial cells of the excretory tubules normal. A few typical hyalin casts. No infiltration whatever of the intersitial tissue.

Experiment 5.—June 28, 1907. 7.30 a. m.

Preparation of the Kidneys of the First Animal.—Middle-sized female cat in good health. Etherization. Exposition and isolation of both kidneys, which are abnormally pale, the left suprarenal, the ureters and the corresponding part of the bladder by the ordinary method. The aorta and vena cava are dissected but not cut. The organs are covered with compresses of Locke's solution.

Preparation of the Host.—Strong, slender, young, black cat. Etherization. Exposition and extirpation of both kidneys by the ordinary method. Dissection of the aorta and vena cava, and temporary hæmostasis. Section of the aorta 2.5 centimeters below the mouth of the renal arteries, resection of the segment of vena cava on which the renal veins are implanted, washing and greasing of the vascular ends.

Extirpation of the Kidneys from the First Animal.—Washing of the kidneys by the ordinary method. Section of the vessels. The anatomical specimen is removed and placed in the abdominal cavity of the second animal. The first animal is killed by opening the diaphragm and clamping the heart.

Transplantation of the Kidneys.—The kidneys are put in their normal location and the vascular segments interposed between the ends of the aorta and vena cava. Anastomoses. Because the incision of the abdominal wall is too short, and consequently the operative field narrow and deep, the anastomoses are difficult and a dissecting forceps is frequently used in the handling of the vascular ends. Reëstablishment of the circulation 45 minutes after clamping the aorta and interrupting the circulation through the kidneys. After a few minutes the kidneys assume their normal appearance; their surface is rosy without white or blue spots. Opening of the bladder and graft of the flap by two planes of sutures.

Suture of the lumbar peritoneum. Through the lower part of the line of suturing an opening is left for the ureters. The intestines are replaced in the abdominal cavity. End of the operation as usual.

June 29. Animal in excellent condition, urinates abundantly.

June 30. Same condition.

July 1. Slight paresis of the posterior limbs.

July 2. Complete paralysis of the posterior limbs and the tail.

July 3. Animal died.

Autopsy.—Peritoneum, kidneys and all abdominal organs apparently normal. Excellent healing of the flap of the bladder. The bladder is distended with urine. Perfect healing of the anastomoses of the vena cava. The upper aortic anastomosis and the renal arteries are in excellent condition. At about 1 centimeter below the mouths of the renal arteries, the aorta is completely obliterated by a clot. This clot is not adherent to the wall of the transplanted segment or to the line of suture. It is fixed to a wound of the intima of the lower end of the aorta at 1 millimeter below the anastomosis. The wound has been caused almost certainly by the handling of the vessel with the dissecting forceps during the performance of the anastomosis.

As the autopsy was performed a long time after death and the temperature was high, the specimens of kidneys are so poorly preserved that no interpretation of the sections is possible.

Experiment 6.[18]—July 12, 1907, 8.15 a. m.

Extirpation of the Kidneys.—Black, pregnant cat, mangy and in bad health. Etherization, extirpation and dissection by the ordinary method, both kidneys, left suprarenal gland, ureters and a large flap of bladder. Clamping of the aorta below the diaphragm at 8.40 a. m. Washing of both kidneys with Locke's solution at the temperature of the laboratory. The perfusion is stopped when there is still a good deal of blood in the kidneys and the veins are still filled with bloody fluid. Then the vessels are cut, the specimen removed and put into Locke's solution.

Preparation of the Host and Transplantation of the Kidneys.—White and black, young female cat in excellent health. Bloody discharge from the vagina. Parturition two days before. Etherization difficult. Semi-circular transverse laparotomy. Evisceration of the intestines, spleen and bladder. Longitudinal incision of the lumbar peritoneum, extirpation of both kidneys, dissection of the

[18] This experiment was reported by Mr. R. D. McClure before the Seventh International Zoological Congress.

aorta and vena cava, and temporary hæmostasis. Resection of the segment of vena cava where the renal veins are implanted. Section of the aorta 2 centimeters below the renal arteries. The anatomical specimen is put into the abdominal cavity, the kidneys in their normal location, and the vascular segment between the ends of the aorta and vena cava. Reëstablishment of the circulation at 9.45 a. m. No leakage. In a few minutes the circulation of both kidneys is apparently normal. The ureters are put along the right side of the rectum through the basis of the right broad ligament. Incision of the bladder and implantation of the flap of bladder by two planes of sutures. Suture of the incision of the lumbar peritoneum. The intestines and the spleen are put back into the peritoneal cavity. Intestines cold, a little shock. Suture of the abdominal wall by the ordinary method. Gauze and cotton dressing. Shirt. Animal in a metabolism cage.

Cat 6 looking at a piece of meat. Photograph taken on the twenty-first day after the operation.

July 12, 2 p. m. Animal a little shocked, lies down in its cage.

July 13. Animal lies down and refuses to eat. From time to time she gets up, turns around in the cage, and cries as though suffering abdominal pain. Does not vomit. After having urinated she is quiet and looks comfortable again. Urine 120 c.c.

July 15. Animal much better. Walks about the cage, no abdominal pain. Eats a great deal of raw meat, drinks milk, urinates abundantly. Bloody discharge from the vagina. No analysis of urine.

July 16. Animal in normal condition, walks, jumps, climbs, eats a great deal of meat, drinks milk, and urinates abundantly.

July 17, 18. Same condition.

July 19. Animal in perfect health, is growing fat. The dressing is removed. Wound completely healed. Both kidneys normal in size and situation.

July 20, 21 and 22. Same condition.

July 23. Animal fat, and in good health. Eats a great deal of meat. Both kidneys are a little increased in size and less movable.

July 24. Same condition.

July 25. Animal is apparently normal, runs about the roof, climbs and jumps on the table, eats a great deal.

ANALYSIS OF URINE: EXPERIMENT 6.

	Quantity.	Color.	Reaction.	Density.	Urea per 100 c.c.	Albumin per 1000 c.c.	
July 13	120 c.c.	Yellowish pale	Acid	1.007	1.2	less than 0.5	
14							Mixed with feces and milk.
15							
16	140 "	Yellowish	Acid	1.015	1.7	0.5	
17	210 "	Yellowish		1.019	from 2.7–4.2	less than 0.25	
18	95 "	Yellow	Acid	1.018		less than 0.5	
19	82 "	Yellow		1.021	4.9	less than 0.25	
20	120 "	Yellow	Acid	1.026	4.1	none	
21	145 "	Yellow	Acid	1.020	4.4	none	
22	95 "	Yellow		1.022		none	
23	164 "	Yellow		1.029	5.1	none	
24							Mixed with feces.
25	170 "	Yellow		1.035		traces	
26	60 "	Yellow	Acid	1.030		0.75	
27	175 "						Mixed with feces or milk.
28	185 "						
29	255 "	Yellowish		1.019			
30	165 "	Yellowish pale		1.013	1.8	1.25	
31	215 "					1.2	
Aug. 1	160 "						
2	165 "						
3							
4							
5							
6							
7	from						
8	120–160 c.c.						
9							
10						A great deal of albumin.	
11							
12							

I am much indebted for this observation to Dr. Levene and Dr. Auer who, during August, had the kindness to analyze the urine of this animal and to examine her clinically.

July 26, 27, 28, and 29. Same condition.

July 30. Animal is apparently in excellent health. Nevertheless, both kidneys are enlarged and increased progressively in size and are completely fixed and adherent to the lumbar region. They are no longer movable in the abdominal cavity as normal kidneys are.

July 31 and August 1. Same condition.

August 2. A photograph is taken while the animal is eating. (See figure.)

August 3. Animal is apparently in good health, but the kidneys are enlarged and the urine contains more albumin.

From August 3 to August 10 the animal was in excellent condition, eating and acting as a normal cat. On August 11 she began to vomit and in a few hours became very ill. A great deal of albumin in the urine. Died on August 12.

Autopsy.—The body was opened by Mr. McClure. The peritoneum, the intestines, the lungs and the heart were found normal. The kidneys and the vessels were not examined, but the body was put in formalin, and the autopsy completed on October 4.

Macroscopical Examination.—Kidneys in their normal location and greatly enlarged. Normal cicatrisation of the flap of the bladder. Both kidneys are strongly adherent to the posterior abdominal wall and to each other. They are united by a growth developed under the peritoneum and passing as a bridge over the aorta and vena cava. This growth is developed outside of the capsule of the left kidney. It is adherent to and interposes itself between the renal vessels, the aorta and vena cava. The vena cava is compressed against the internal face of the right kidney. The right renal vein is also compressed between the tumor and the kidney, while the left renal vein is too much extended. This growth is composed of hard, white, apparently fibrous tissue, well-defined on its anterior side and more diffuse on its posterior side. It is intimately adherent to the transplanted suprarenal gland. Anastomoses excellent. Opening of the kidneys. No hydronephrosis. Congestion. Dilatation of the stellate veins.

Microscopical Examination.—Cadaveric changes of the epithelial cells are so marked that an interpretation of the epithelial lesions is not possible. Glomeruli well preserved, dilatation of the capillary loops which fill almost completely the capsules. Infiltration by plasma cells of the interstitial tissue. This lesion is more marked in the cortex than in the medulla. Focal disposition. Very marked dilatation of the blood-vessels. Appearance of acute interstitial nephritis. Section of the growth shows organized blood clot.

Experiment 7.—July 17, 1907.

Extirpation of the Kidneys.—Pregnant female cat. Etherization. Preparation by the ordinary method of both kidneys, left suprarenal gland, vessels, ureters and flap of bladder. Incomplete perfusion of the kidney as in the previous experiment. Extirpation of the anatomical specimen, which is put into Locke's solution at the temperature of the laboratory (22.2° C.).

Transplantation of the Kidneys.—Young, black, pregnant cat in excellent health. Abdomen considerably enlarged; near the end of pregnancy. Semicircular transversal laparotomy. Evisceration of the intestines, the spleen, the bladder filled with urine, and the uterus, which is distended by several fœtuses. Ligation of the left ovarian vein. Longitudinal incision of the lumbar peritoneum

in the middle line and extirpation of both kidneys. Dissection of the aorta and vena cava, and temporary hæmostasis with *serre fines.* The anatomical specimen is placed in the abdominal cavity by the ordinary method. Reëstablishment of the circulation 60 minutes after the interruption. No leakage. Excellent and quick reëstablishment of the circulation through the kidneys which assume almost immediately a normal color. Incision of the bladder and graft of the flap of bladder by the ordinary method. After completion of the suture, it is observed that the ureters are twisted around each other. However, the transplanted ureters being very long there is no tension and it will not interfere, probably, with the flow of urine. The intestines, the spleen and the uterus are put back into the abdominal cavity, which is closed by the ordinary method. Gauze and cotton dressing. Shirt.

July 18. Animal lies down, urinates abundantly and looks in good condition.

July 19. Parturition normal. No eclampsia. Urinates and is in good condition.

July 20. Animal apparently normal, walks, drinks milk and eats meat. Bloody discharge from the vagina.

July 21, 22, 23, and 24. Almost the same condition.

July 25, 26. Animal less well, urinates and eats a little meat. High temperature.

July 27, 28. Animal refuses to eat, and looks very ill. However, she urinates abundantly. Bloody discharge from the vagina.

July 30. Animal died.

Autopsy.

Macroscopical Examination.—Large abscess of the pelvis, located on the left side, between the pelvis and the rectum, extending as far as the subperitoneal space, and opening to the skin near the anus, on the left side. Big and soft uterus. The left uterine horn is increased in size. On section reddish fluid and a part of a placenta is found.

No peritonitis. Intestines normal. Both kidneys surrounded by loose connective tissue, normal in location, color and consistency. Anastomoses and vessels normal. Slight congestion of the pyramids. No dilatation of the ureters. Excellent healing of the bladder.

Microscopical Examination.—Glomeruli well preserved. No coagulated fluid between the capsules and the capillary loops. It is difficult to appreciate exactly the lesions of the epithelium of the tubules, for the fixation of the specimen is not good. It seems well enough preserved. Slight leucocytic infiltration between certain tubules. Slight dilatation of the blood-vessels.

Experiment 8.—July 19, 1907.

Extirpation of the Kidneys.—Middle-aged pregnant cat. Dissection of the anatomical specimen by the same method as in the preceding experiments. A small quantity of Locke's solution at the temperature of the laboratory (31° C.) is injected into the kidneys. The washing is stopped when the renal vein is still filled with bloody fluid. Anatomical specimen removed and put in Locke's solution at the temperature of the laboratory. Cat killed by hæmorrhage.

Transplantation of the Kidneys.—Long, middle-aged male cat. Semi-circular transversal laparotomy. Evisceration of the intestines and the spleen. Extirpa-

tion of both kidneys. Dissection of the aorta and vena cava, and temporary hæmostasis. Section of the aorta below the renal arteries. Section of the vena cava between the openings of the renal veins. The kidneys are removed from their jar, put into the abdominal cavity and the vessels anastomosed by the ordinary method. Reëstablishment of the circulation 1 hour and 5 minutes after the interruption. No leakage. Excellent circulation through the kidneys, which assume, after a few minutes, their normal appearance. Opening of the bladder and graft of the flap of bladder. Suture of the lumbar peritoneum. It is found that the right ureter goes behind the vena cava and produces a slight degree of compression of this vessel. The intestines are put back into the abdominal cavity. Closing of the abdominal wall by three planes of sutures. Gauze and cotton dressing. Shirt.

July 20. Animal in excellent condition. Urinates abundantly.

July 21, 22. Animal drinks milk.

July 23. Animal apparently in normal condition, walks about the roof. The dressing is removed. Apparent healing of the wound.

July 24. Same condition.

July 25. Animal eats raw meat.

July 26, 27 and 28. Animal in good condition, eats meat.

July 29. Animal is less well. An abscess has developed on the left side below the end of the abdominal suture.

July 30. Animal looks ill. However, he urinates as usual.

July 31. Animal refuses to eat, and looks very ill. The abscess is examined. It is found that it is an extensive and deep abscess of the wall itself, and not merely of the subcutaneous tissue. All the left part of the abdominal wall is involved. Large openings and drainage. In the evening the animal is worse.

August 1. Animal died in the morning.

Autopsy.

Macroscopical Examination.—Large abscess infiltrating the anterior and left parts of the abdominal wall. No peritonitis. Heart, lungs, liver, and spleen normal. Both kidneys are almost normal in size, color, and consistency, and surrounded by a layer of adipose tissue, which has become very abundant below the lower end of the kidneys. Their mobility is normal. Both ureters adherent to one another enter the peritoneal cavity at the level of the lower end of the incision of the peritoneum near the lower end of the left kidney. Perfect union of the transplanted flap to the bladder.

Opening of the bladder. On the posterior wall the flap is found limited by a linear and almost circular scar. The transplanted mucous membrane is normal. The mouth of each ureter presents its ordinary appearance.

Opening of the lumbar peritoneum and dissection of the vena cava. No perivascular sclerotic tissue. The right ureter is twisted around the vena cava. Renal veins almost horizontal, dense connective tissue around the left suprarenal gland. Opening of the vena cava, anastomoses perfectly smooth. A little clot around and in which a short piece of silk thread is found free in the lumen of the vein at 1 centimeter above the upper anastomosis. In a point of the upper anastomosis is a very small red spot where this clot was probably adherent. Dissection of the aorta. The lower anastomosis is almost invisible: at the level of the upper anastomosis, the aorta is adherent to the vena cava by

dense connective tissue. This tissue is removed and, corresponding to the point where it is mostly adherent to the wall of the aorta, a very small opening of the line of sutures between two stitches is observed. The aorta is opened: anastomoses smooth and glistening.

Longitudinal section of the kidneys: cortex and pyramids almost normal although a little congested. A small piece is fixed in Zenker's fluid.

Microscopical Examination.—Glomeruli well preserved. Epithelium of the tubules without very marked lesions. Very few casts. Extensive foci of infiltration of the interstitial tissue by plasma cells; subacute interstitial nephritis. Slight dilatation of the blood-vessels.

Experiment 9.—October 14, 1907.

Dissection and Preparation of the Kidneys.—Middle-aged female cat. Etherization. Preparation of the anatomical specimen as usual. The lower suprarenal vein is ligated and the gland is not included in the specimen. Section of the uterus and the intestine. The specimen, of which the circulation is not interrupted, is protected by the omentum and towels.

Preparation of the Host.—Gray and white female cat, living in the laboratory several months; young and in good health. October 1. Urine examined—yellow, clear urine. Density 1.039. Urea 5.9 gr. per 100 c.c. No albumin. Etherization. Opening of the abdomen and evisceration as usual. The intestines are protected by Japanese silk towels sterilized in vaseline. Dissection and extirpation of both kidneys. Ligature of the ovarian veins, of the lower suprarenal vein and of two aortic collaterals.

Extirpation of the Kidneys.—Clamping of the aorta below the diaphragm at 11.25 a. m. Incomplete washing of the kidneys with Locke's solution. Section of the vessels and removal of the specimen.

Transplantation of the Kidneys.—The specimens are placed immediately in the abdominal cavity of the host. After temporary hæmostasis with *serre fines,* the aorta is cut 3 centimeters below the renal arteries. Resection of the region of implantation of the renal veins. Washing and greasing of the vascular ends. Anastomosis of the vessels. Reëstablishment of the circulation at 12.15 p. m. No leakage. Excellent circulation through the kidneys and the small vessels of the lower ends of the ureters. Nevertheless, the left kidney remains pale, while the right kidney is rosy and secretes urine. Opening of the bladder and graft of the flap by two planes of stitches. After the completion of this suture, a slight hæmorrhage from the lower arterial anastomosis is noticed and controlled by one stitch.

The appearance of the kidneys is now very much modified. The left kidney is vasodilated, and its vein carries red blood, while the right kidney has become pale and vasoconstricted, and its vein is filled with dark blood.

End of the operation as usual.

October 14, 4 p. m. No shock. Animal walks, drinks water, has urinated 9 c.c. of bloody urine, the blood depositing itself quickly at the bottom of the glass, urine clear on the top. Urea 4.9.—11 p. m. Urinates again, much less blood.

October 15. Animal a little sick, drinks water, walks about the cage.—4 p. m. Since yesterday 25 c.c. urine only, dark yellow, with a little blood. Albumin. Density 1.051.

October 16. Animal in better condition, drinks milk and eats a little meat. Urine 16 c.c.

October 17, 18. Good condition, drinks milk, is given very little meat.

October 18. Same condition.

October 19. Cat in excellent condition. He is given plenty of milk and very little meat.

October 20. Same condition.

October 21. Cat is completely recovered. He is a normal cat, in the same condition as before the operation.

October 22. Diet from now raw liver and milk.

October 23. Dressing is removed; wound completely healed.

October 24. Cat normal. By palpation the kidneys are found small and movable. They are apparently normal.

October 25. A sample of urine is examined. Clear, yellow urine, acid, density 1.030. Urea 5.1. No albumin.

October 26. Cat normal, kidneys normal in size and movable.

October 27. Same condition.

October 28. Cat being normal is allowed to go out from the cage and to run freely through the room.

October 29. Cat is put into another room and spends all day climbing on and jumping off the furniture.

October 30. Normal general condition, kidneys a little enlarged and less movable.

October 31. Cat a little depressed. The kidneys are very much enlarged and fixed to the lumbar wall. Samples of urine are then examined.—1 p. m. Pale yellow, clear urine. Urea 3.6. Marked quantity of albumin.—3 p. m. Heavy precipitate of albumin by nitric acid.—6 p. m. Albumin 6 gram per 1000 c.c.

November 1. Cat is a little depressed, but still in very good general condition. The kidneys are much enlarged. Very large amount of albumin.—10.30 a. m. Etherization. Semi-circular transversal laparotomy, just above the scar. A few adhesions of the omentum to the wall. Peritoneum and intestines normal. Both kidneys appear very much enlarged and covered with sound peritoneum. Their consistency is a little softer than normal. Incision and dissection of the lumbar peritoneum on the middle line. There is a little sclerosis of the subperitoneal connective tissue around the anterior side of the vena cava. It may possibly produce a slight degree of compression of the vessel. Nevertheless, the arterial and venous circulation appear to be normal. The connective tissue of the hilus is œdematous, clear fluid flows after incision. The wall of the ureter is œdematous, without congestion. The small vessels are distinctly seen with red blood. The color of both kidneys is rosy and normal. There is no congestion. Incision of the capsule of the right kidney: clear fluid and red blood flow. The tissue of the kidney is incised: it is œdematous and not congested. Abundant hæmorrhage. Suture of the capsule with Lyon's silk and needles, No. 16. Both renal veins of the right kidney are dissected. The circulation is normal. The perivascular connective tissue is not sclerotic but œdematous. During the dissection of the upper vein several blue, round spots are seen on the surface of the organ, and disappear after a few minutes. No suture of the incision of the lumbar peritoneum. Abdominal wound closed as usual.

6.30 p. m. Cat in good condition. Urine 18 c.c. Much less albumin, 2.75.

November 2, 8 a. m. Cat in good condition, but depressed. Walks about cage. Takes milk only, possibly a little meat.—10.30 a. m. Urine 19 c.c. Albumin 0.60.

9 p. m. Urine 53 c.c. Density 1.020. Albumin 1.50. Urea 4.2.

November 3. Same condition. Diet consists only of milk. Cat depressed.

November 4. Better condition. 8 a. m. Urine 61 c.c., dark yellow, density 1.033. No albumin.

November 5. Same condition.

November 6. Albumin again 1.50.

November 7. Cat depressed, walks about its cage.

November 8. Better condition. Eats a little fish.

November 9. Cat in better condition, but very emaciated. Therefore, he is given rare liver and codfish and eats hungrily. Dressing removed. Wound healed. Both kidneys much diminished in size, but still abnormally large.

November 10. General condition improved.

November 11. Both kidneys diminish steadily in size. Cat eats codfish.

November 12. Cat eats meat and fish. From 9 a. m. to 6.30 p. m., urine 48 c.c., clear yellow. Density 1.021. Urea 3.2. Albumin 1 gr.

November 13. Cat in good condition. Albumin less than 1 gr.

November 14. Cat well in the morning. In the evening, looks ill. Discharge from the nose. Refuses to eat.

November 15. Abundant nasal discharge. Cat refuses to eat. Urine, yellow clear. Albumin less that 1 gr.

November 16. Animal weak and emaciated. Very abundant purulent nasal discharge. The quantity of urine during the last twenty-four hours was 78 c.c., yellow. Density 1.031. Albumin 1.80. Urea 4.8. Many red blood corpuscles and granular casts. The size of the kidneys almost normal.

November 17. Animal very emaciated, weak, but still able to jump from his cage and walks about the room. Abundant nasal discharge. Refuses to eat.

November 18. Animal very weak.

November 19. 1 p. m. died. Post-mortem 2.15 p. m. Opening of the abdominal cavity. A loop of jejunum is adherent to the posterior abdominal wall. Sharp flexure without obstruction. Both kidneys normal in location, color, size and consistency. Ureters normal. Perfect healing of the transplanted flap of bladder. In the bladder, yellow clear urine 5 c.c., albumin. Longitudinal incision of the kidneys, which are apparently normal. However the cortex is pale. Medulla normal. Capsule normal. No dilatation of the stellate veins.

The main pathological change is a general and intense calcification of the arterial system. The arteries are as hard and friable as thin-walled glass tubes. These lesions have developed since the transplantation, for the cat was a young animal in excellent health and its abdominal aorta was perfectly normal.

V. RESULTS.

The results of the experiments will be examined from the clinical and anatomical standpoints.

A. *Clinical Results.*

In every case the reëstablishment of the renal functions was observed. These functions were determined by the characters of the urines and the general condition of the animals.

The secretion of urine may begin as soon as the arterial circulation is reëstablished. In several cases, clear urine flowed from the ureters while the flap of bladder was being grafted onto the host. More often, no urine was seen flowing from the ureters immediately after completion of the operation. But the secretion always began during the first twenty-four hours. It is difficult to ascertain exactly the amount of urine secreted during the first few hours, because of the vomitus and water which are often mixed with urine. However, Cat 6 did not vomit, and after the first twenty-four hours the jar contained 120 cubic centimeters of urine. On the other hand Cat 1 urinated very little on the first day. Cat 9 urinated only 25 cubic centimeters during the first twenty-four hours; the second day the amount of urine passed was only 16 cubic centimeters, this urine was highly concentrated and contained much urea. These differences in the immediate amount of urinary secretion are due probably to unknown conditions of the vasomotor nerves. It is generally supposed that denervation of the kidney produces the secretion of an abundant and diluted urine. In the simple transplantation of the kidney, when, for instance, an isolated organ is transplanted into the neck, these phenomena were observed. But in the case of transplantation in mass, immediate vasodilatation is not so marked. Sometimes there is vasoconstriction, but oftener the kidneys retain their normal appearance. Exceptionally, vasodilatation alternates with vasoconstriction. In Experiment 9 about ten minutes after the reëstablishment of the circulation, the right kidney was rosy, its venous blood red, and some urine flowed from its ureter, while the left kidney was pale, and apparently did not secrete. About thirty minutes afterwards, when the suture of the bladder was completed, both kidneys were examined again. The conditions were now reversed: the right kidney had become pale, and its vein filled with dark blood, while the left kidney was rosy and its vein contained red blood. It seems

that following transplantation the renal ganglia begin to act and that variable conditions of the nervous system may be responsible for the differences in the immediate results observed.

In all the experiments, the urinary secretion went on as long as the animal lived. Every cat urinated abundantly every day. But the animals presented sooner or later some complication, which modified in some measure the renal functions. As is to be expected after an operation as complex as the transplantation in mass, various accidents occurred; hydronephrosis, intestinal compression by peritoneal adhesions, volvulus, phlegmon, puerperal infection, compression of the renal veins by organized hæmatoma of the connective tissue, which were the direct or indirect causes of death in these animals. It is well known that several of the complications, especially the compression of the renal veins, produce grave renal lesions of their own. Therefore, the results of our experiments must not be considered as expressing generally the normal condition of transplanted kidneys, but merely of transplanted kidneys when subjected to various complications, that is, of more or less abnormal transplanted kidneys. Actually, it is impossible to know exactly how a normal transplanted kidney would functionate, for we cannot as yet discriminate between the disorders produced by such a common complication as hydronephrosis or compression of the veins and the less defined ones which may be due to lesions produced by the transplantation itself. However, in Experiment 6 and 9, for instance, the functions of the kidneys seem to have been for a certain time almost completely normal.

The color of the urine was yellow, generally or often less dark than the normal urine of the cat. Its reaction was acid. Its quantity for twenty-four hours oscillated between 120 and 160 cubic centimeters. But it might be, exceptionally, 25 and even 15 cubic centimeters, or, in another case, 215 or 255 cubic centimeters for twenty-four hours. In this case there was congestion of the kidneys produced by venous compression. The density was very far from constant; generally it oscillated between 1.018 and 1.030, going sometimes as high as 1.035 and 1.051. In Experiment 6 there was little parallelism between the amount of urine and the

density. Once the kidneys secreted 170 cubic centimeters of urine with a density of 1.035.

In all cases the amount of urea bore a relation to the diet of the animal. In Cat 6, abundantly fed with raw meat, the amount of urea varied from 2.7 to 5.1 grams. Cat 9 passed through his own kidneys, fourteen days before the operation, 5.9 grams of urea for 100 cubic centimeters. Eleven days after the operation, he eliminated through his new kidneys 5.1 grams of urea for 100 cubic centimeters. The difference is explained by the diet which was less abundant after the operation than before.

Among the abnormal constituents of the urine, the presence of albumin only has been looked for. In Experiment 1 albumin was present during the fourteen days of the post-operative life of the animal. These kidneys were abnormal owing to the perfusion with too hot Locke's solution and to a developed hydronephrosis. In the other cases there was little albumin during the first days, ranging from 0.50 to 0.25 gram for 1000 cubic centimeters. This was probably due in part to the blood coming from the suture of the ureter or the bladder. The amount of albumin decreased progressively and disappeared about one week after the operation. In Experiment 6 albumin was again found on the thirteenth day after the operation, and its amount increased progressively to 1.50 grams and beyond. In Experiment 9 there was albumin in the urine one day after the operation. On the eleventh day no albumin at all was present. On the fifteenth and sixteenth days the animal was allowed to run and climb freely. On the seventeenth day albumin was found again in marked quantity and, at the same time, enlargement of the kidneys was distinctly detected by palpation.

The general condition of the animal can be used, in some measure, to indicate the perfection of the urinary elimination. As long as no complications were present, the animals lived as normal cats do, without presenting any symptoms which could be considered as produced by renal insufficiency. When general complications occurred the cats reacted against them in normal ways.

Cat 1 suffered from eventration, due to a premature resorption of the catgut in the abdominal suture. In two other animals sutured with the same catgut, the resorption and eventration

occurred on the fifth or on the seventh day after the operation. We may admit that in Experiment 1 the resorption took place at about the same time. However, in spite of the extrusion of intestine outside of the abdominal cavity in the gauze dressing, the animal lived several days, drank water and milk and ate a little meat, and urinated abundantly. When the reduction of the inflamed intestines into the abdominal cavity was performed, fourteen days after the operation, the animal was still able to overcome the operative shock, and to walk about the room a few hours later.

In Experiment 6 the animal was in apparently normal condition four days after the operation. She walked about the room, played and ate a great deal of raw meat. Her condition remained excellent for several weeks. Twenty days after the operation she was in good health, had glossy hair, was very fat and ate with appetite all kinds of food. She ran about the room, played, jumped and climbed on the desks and tables as a normal cat does (see figure). There was, however, albumin in the urine, and slow and progressive enlargement of the kidneys took place, which showed that she was not in an entirely normal condition. Nevertheless, until the twenty-ninth day after the operation, she seemed to be in excellent health. Then gastro-intestinal symptoms appeared and death occurred on the thirty-first day after the operation. In Experiment 7 the animal operated on was a pregnant cat whose uterus contained several large fœtuses. After the operation, she was immediately in good condition. Two days afterwards parturition occurred without any eclamptic fits or any abnormal symptoms. As the animal seemed to recover very easily and began eating meat one day afterwards, she was not observed very carefully. The dressing was removed and she was let alone. When she was examined again, eight days after the operation, she was found less well and feverish. Her condition grew worse and she died thirteen days after the operation. The autopsy showed puerperal infection with retention of a placenta and an enormous abscess of the pelvis.

Experiment 9 was a female cat which lived in the laboratory for several months. She was in excellent condition when she was operated on, and recovered very quickly from the operation. Her life went on just the same as before. The kidneys were movable

and small. She looked in excellent health and lived as a normal cat. On the eighteenth day after the transplantation a direct examination of the kidneys was made to ascertain the cause of the appearance of albumin. The general condition was little affected by the operation and the albumin disappeared on the twenty-first day, but reappeared again a little later. On the thirty-fifth day, the animal was very weak and emaciated. She died on the thirty-sixth day.

We can conclude from these results that the functions of the kidneys reëstablish themselves after the transplantation. Since an animal, such, for instance, as Cat 6, can live in an apparently prospérous condition of health fifteen or twenty-five days, and more, after a double nephrectomy, and eliminate each twenty-four hours 120 and 160 cubic centimeters of urine through the new kidneys, it is certain that the functions of the transplanted organs are efficient. Even these functions during a part of the life of animals No. 6 and 9 can be considered as having practically been normal. When complications contingent or inherent to the actual method of transplantation occurred, the functions of the kidneys were modified and became abnormal according to the pathological changes suffered by the organs.

B. *Anatomical Results.*

The Blood Vessels.—The condition of the blood vessels was examined three times by laparotomy on the living animal and in the other cases at the autopsy..

The direction of the vena cava was almost always found normal. Once, however, the interposed segment was too long and bent. In Experiment 9 the position of the veins was modified considerably by an organized hæmatoma which had pushed the vena cava against the right kidney. The right renal vein was compressed and the left one too much extended. This diminished the activity of the venous circulation and produced marked congestion of the kidneys. It is very important that the veins be given their normal situation and direction. On account of the low pressure and the thinness of the wall, they are not able to take care of themselves as arteries do. Every departure from the normal produces a diminution of

the caliber and consequently slight or marked congestion of the transplanted organ.

The relations of the veins with the surrounding structures, arteries and ureters were generally normal. It happened once that by mistake the right ureter was twisted around the lower part of the venous segment. It did not appear to cause any marked disturbance. The vessels were free in loose connective tissue, excepting in Experiment 6 where the vena cava and renal veins were compressed by an organized hæmatoma. When progressive compression of the veins had occurred it was expressed clinically by progressive enlargement of the kidney, and by reappearance of albumin in the urine on the thirteenth day after the operation, while the animal appeared otherwise in perfect health. In another case there was a little sclerosis of the perivascular connective tissue, which produced some retraction of the right kidney toward the middle line. The induration or sclerosis of the connective tissue may be a serious secondary complication. It occurs in the transplantation in mass but more often in simple transplantation, and oftenest in transplantation with implantation of the renal vessels on the aorta and vena cava. The induration has no influence on the arteries, but interferes with the venous circulation. As soon as the vein is no longer able to dilate freely in the loose connective tissue of the hilus, its circulation is slightly hampered and the result is a chronic congestion of the organ. This sclerosis may be brought about also by slight non-suppurative inflammation or perhaps by chemical irritation. It is probable that this condition is often due to a slight infiltration of blood into the connective tissue. Blood has an irritative influence on tissues. In Experiment 8 there were strong adhesions between the aorta and vena cava, at the level of the upper arterial anastomosis. The vessels were connected by a mass of dense connective tissue. The aorta was dissected, and the maximum of adhesion was found to be on the anastomosis itself and on this point there was a small gap between two stitches. The connective tissue was probably produced under the influence of the infiltration of blood through this opening. Many examples of this sclerotising influence of blood have been observed after transplantation of segments of vessels. It is well

known that extravasation of blood in joints, muscles, or in the central nervous system produce hard connective tissue. Thus injection of blood has been utilized by Bier and Schmieden[14] for inducing callous formation in cases of pseudarthrosis. The perivenous sclerosis, which is a dangerous secondary complication in transplantation of organs, can be prevented probably by rigid hæmostasis and asepsis.

The venous anastomoses healed without thrombosis or stenosis. In one case there was an obliteration of the vena cava due to a torsion of the vein. But this was quite independent of the anastomosis. In Experiment 8 the venous anastomosis was normal. Nevertheless, a very small ovoid clot, which developed around a fragment of silk thread, was found free in the lumen of the vein, about one centimeter above the upper anastomosis. On the anastomosis itself by minute examination a little red spot was detected, which might have been the point where this clot was adherent. This is an absolutely exceptional complication.

The aorta and the transplanted aortic segment assumed in every case a normal direction and appearance. The direction of the renal arteries was the reverse of normal. This was due to the fact that the transplanted segment was fixed on the aorta below the implantation of the normal renal arteries. A modification of direction has no harmful influence on the arterial circulation. The thickness of the wall and the high blood pressure allow the arteries to adapt themselves to abnormal situations. Only one complication was observed: in Experiment 5 there was a complete obliteration of the lower part of the venous segment and the lower end of the aorta by a thrombus. This thrombus was dissected and found adherent to a wound of the intima, just below the lower anastomosis. The wound was evidently produced by the dissecting forceps used in this case for handling the vessel.

The anastomoses healed without thrombus or stenosis. The intima of the transplanted segment was smooth and glistening. No deposit of fibrin was observed, and consequently no embolus. In Experiment 1 a fatty embolus was noticed. A few minutes after the reëstablishment of the circulation, the right kidney assumed

[14] Schmieden, *Jour. of Amer. Med. Assoc.*, 1907, xlviii, 395.

a rosy and normal color, while the left kidney remained pale. The lower end especially was almost completely yellowish white. An incision through the capsule was made at this point. A small hæmorrhage of red blood mixed with a good deal of vaseline followed, and stopped after a few moments. However, the circulation of the left kidney improved progressively and after thirty minutes was almost normal. It is probable that the vaseline under the progressive increase of temperature of the kidney became more fluid and flowed through the capillaries. The anatomical examination of the kidneys, fifteen days after the operation, showed no evidence of this embolus. The few accidents described could be almost completely prevented by operating on animals of a larger size.

The Nervous System.—The anatomical conditions of the nervous system of transplanted kidneys are not yet known. The attempt was made to preserve as completely as possible the circulatory apparatus of the nervous ganglia with the hope that they would resume their functions partially. It is not impossible to believe that sympathetic ganglia of which the vessels are respected and the circulation reëstablished immediately after the transplantation do not degenerate completely. It has been shown, especially by the experiments of Morat,[15] that sympathetic nerves have some of their trophic centers in the ganglia. A part of the vasoconstrictor nerves of the tongue, contained in the hypoglossus nerve, have a trophic center in the superior sympathetic ganglion. After section and degeneration of the sympathetic, stimulation of the hypoglossus still produces vasoconstriction of the tongue. After extirpation of this ganglion, stimulation of the hypoglossus becomes negative. After intra-cranial section of the facial nerve on a dog, almost all vasomotor fibres degenerate from six to twenty-six days after the operation. However, the stimulation of the chorda tympani produces a slight vasodilatation of the gland. Morat assumes that in this case the geniculatus ganglion must be considered as a trophic center for these fibers.

Even if it be admitted that nervous ganglia can, in some measure, functionate when severed from the central nervous system, it is

[15] Morat, *Comp. rend. de l'Acad. des sciences,* 1897, cxxiv, 1389.

not certain that transplanted ganglia can recuperate their functions. The experiments of Stewart and Guthrie[16] show that after an acute and complete anæmia of the nervous centers for more than twenty minutes, the reëstablishment of their functions is not possible. The functional activity of the ganglia should necessitate also the reestablishment of a practically normal circulation. The experiments of Tuckett[17] have demonstrated that if the vascular supply of the upper sympathetic ganglion is deranged, degeneration sets in forthwith. But, by preserving the connective tissue surrounding the pedicle of the kidneys and not cutting the small collateral branches of the aorta and vena cava, the vascular apparatus of the kidneys can possibly be kept in its integrity. In this condition, the ganglia may resume their functions, if it be physiologically possible.

Ureters and Bladder.—Dilation of the ureters and hydronephrosis took place in the cases where anastomoses of the ureters were performed. In Experiment 3 the upper part of both ureters was very much dilated and the invaginated part stenosed. However, the urine flowed into the bladder satisfactorily, as is shown by the clinical history of the animal. The ureters of a cat are so small that the anastomosis is very difficult, and stenosis or disunion occurs. On small animals it seems proper to give up completely uretero-ureteral anastomoses.

The results of the graft on the bladder of the host of a fragment of bladder extirpated around the points of implantation of the ureters were excellent. The anatomical specimens showed that there was no distension of the upper part of the ureters or the kidneys. Both ureters, adherent to one another, entered the peritoneal cavity through the lower part of the incision of the lumbar peritoneum and went downward along the right side of the rectum. In one case they were twisted around one another; in another, the right ureter was twisted around the vena cava. In spite of these faults of technique the functional and anatomical results were very satisfactory.

In every case, the union of the flap of the bladder of the host took place. After opening the bladder, the transplanted flap ap-

[16] Stewart, Guthrie, Burns and Pike, *Jour. of Exper. Med.*, 1906, viii, 289.
[17] Tuckett, *Jour. of Physiol.*, 1905, xxxiii, 77.

peared congested and swollen, or entirely normal with the same color and appearance as the surrounding mucosa from which it was separated by a linear scar. On the surface of the transplanted mucosa both openings of the ureters were distinctly seen, normal in size and appearance.

The Kidneys.—The kidneys were examined three times only on living animals. During the operations performed on Cats 1 and 9, fourteen and eighteen days after transplantation, the kidneys were seen covered with sound peritoneum and as regards their color, situation and general appearance, they looked just like ordinary hydronephrotic or œdematous kidneys. In other cases, the anatomical examination was performed after the autopsy. For this part of the work, I am very much indebted to Dr. Simon Flexner, who had the kindness to look over the specimens and histological sections and to give me the invaluable help of his advice.

Macroscopical Examination.—In the experiments where there was no hydronephrosis or venous compression, the size of the kidneys was normal. Hydronephrotic and congested kidneys had their ordinary appearances. Their location was always normal. They remained at the place where they were put during the operation and maintained by the suture of the lumbar peritoneum. As a rule, they were not as movable as the cat's kidneys are normally. However, in Experiments 6 and 7 for instance, their mobility was practically normal. The kidneys of Cat 6 were strongly united to each other and to the lumbar wall by hard connective tissue. In the cases where no hydronephrosis or congestion took place, the consistency of the organ was normal. The external and internal appearance of the organs presented no special characters. They looked like congested, hydronephrotic, or almost normal kidneys. The kidneys of Cat 6 were very much congested. The capsule was slightly adherent to the parenchyma. The stellate veins were very much dilated and the medulla and cortex much increased in size.

In Experiments 1 and 3 the urine flowed from the organs when opened, the calices and pelves were dilated, and the surface of the parenchyma congested. The kidneys were ordinary hydronephrotic

organs. In the other experiments, the kidneys assumed the appearance of normal or slightly congested organs.

Microscopical Examination.—The specimens were generally fixed in Zenker's fluid and stained in hæmatoxylin and eosin. Some of them were taken from the animal several hours after death. In Experiments 6 and 7, the body was simply opened and put in a jar of formalin, while the pieces for histological examination were cut from the kidneys two months after. These faults of technique explain why in several cases the specimens were so badly hardened and why there were such cadaveric changes, especially in the epithelium of the tubuli contorti that an interpretation of the pathological lesions was difficult. It was found that the kidneys presented some lesions, very slight in some cases and more marked in others. In Experiment 4, for instance, the glomeruli and the epithelia of the tubules were very well preserved. There was no interstitial infiltration, and a few casts only were observed. The lesions of these kidneys were very slight.

The lesions noticed in the other experiments belong to two classes; hydronephrosis and nephritis. Hydronephrotic lesions were observed in Experiments 1 and 3. The excretory tubes were very much dilated. There was, too, some dilatation of the tubules of which the epithelium was flattened. In a few places between the tubes slight interstitial infiltration was present. In Experiment 3 the glomeruli were normal. In Experiment 1 some coagulated fluid was seen between Bowman's capsule and the capillary loops. The changes of the epithelium of the tubules were slight. The cells were regularly disposed inside the basement membrane, and the brush border was distinctly seen. The lumen contained some fluid exudate, but very few casts were observed. All these lesions may be explained by the presence of hydronephrosis.

Inflammatory lesions were present in three cases; very slight in Experiment 7, but more marked in Experiments 6 and 8. The epithelial degeneration was not extensive in Experiment 8. It seemed more marked in the other cases, but the cadaveric changes were so pronounced that no accurate interpretation was possible. The characteristic lesion met with in these three cases was the infiltration of the interstitial tissues between the tubules. The most

marked case was Experiment 8, in which the foci of infiltration were extensive. The infiltration was composed of cells having the characters of the plasma cells described by Councilman[18] in acute interstitial nephritis, so-called.

This subacute interstitial nephritis is not a necessary complication of the transplantation, since it was absent from the first cases. It is due probably to secondary causes, physical or chemical conditions of the fluid used in the perfusion, congestion of the organ, diet, general condition of the animal, etc. Many factors may come into play during and after the transplantation for injuring the kidneys. The interstitial and epithelial lesions are due doubtless to some of these. It was regarded as surprising that comparatively few changes were found in the renal structure considering that the organs had been exposed to the rough handling of the transplantation. The cells of the secretory epithelium of the kidney are extremely delicate and sensitive to the modifications of the circulation, etc. It is well known that temporary ligature of the renal vein produces extensive degeneration of the epithelial cells. The simple suspension of the circulation has a harmful influence on the epithelium. The cadaveric disintegration of the cells of the tubules begins very early. One hour after death, the brush border has almost always disappeared.

In the transplantation, the renal tissue is not only deprived of circulation for one hour at least, but is also subjected to a perfusion with a fluid which exerts probably its own harmful influence. The perfusion of the organs seems necessary for preventing the formation of clots and the occurrence of thrombosis of the vessels or infarcts of the kidneys. The solution employed is the ordinary Locke's solution. It has been chosen because it is a physiologically balanced fluid. Pure sodium chloride solutions have injurious effects on the tissues. Ringer has shown that minute amounts of calcium and potassium salts antagonize the effects of the pure sodium salt. Loeb[19] and his pupils have laid special emphasis on the poisonous effects of pure sodium chloride solution. Fundulus eggs put in a pure sodium chloride solution of the same concentration as sea

[18] Councilman, *Jour. of Exper. Med.*, 1898, iii, 393.
[19] Loeb, *Amer. Jour. of Physiol.*, 1899–1900, iii, 327.

water cannot live, but they can live if a definite proportion of calcium chloride be added. Howell[20] and Harvey Cushing[21] also showed the injurious effects on the heart and muscles of the pure sodium chloride solution. In their experiments on tumors, Flexner and Jobling[22] found that the percentage of successful transplantations is much higher when the fragments of tumor have been preserved in Ringer's instead of salt solution. Therefore, in the perfusion or washing of delicate anatomical structures, physiologically balanced solutions must always be used. But even such a solution is harmful if it has not the same osmotic tension as the tissues. It is very probable that the osmotic tension of Locke's solution is not exactly suited to the cat's kidney. Rathery[23] has shown that slight variations of the cryoscopic point of the solution in which a fragment of the kidney is preserved is able to modify in a large measure the morphology of the cells. For the rabbit's kidney, the best solution has a cryoscopic point of —0.78 C. All other solutions are nephrolytic. If, for instance, a solution which freezes at —0.90 C. is used, the cells are found retracted and as having expelled into the lumen of the tubules a great many of their nuclear granulations. In order to prevent the osmotic disturbances that Locke's solution probably produces in some measure on the cat's kidney, it would be necessary to determine accurately the cryoscopic point of the solution isotonic for the cat's kidney, and to use then a balanced solution of this same tension.

Nevertheless, even an iso-osmotic, physiologically balanced solution would not be able to keep the kidney in its normal condition. Salkowski[24] has shown that organs kept at body temperature under conditions which prevent bacterial growth undergo self-digestion. The autolysis of liver and kidneys, etc., is due to proteolytic enzymes which are contained in the cells and come into play as soon as the circulation is stopped. Opie[25] succeeded in isolating two proteolytic

[20] Howell, *Amer. Jour. of Physiol.*, 1898, ii, 57.

[21] Cushing, *ibid.*, 1901–02, vi, 77.

[22] Flexner and Jobling, verbal communication.

[23] Rathery, Le tube contourné du rein, étude histologique, anatomopathologique experimentale, Thèse de Paris, 1905.

[24] Salkowski, *Zeit. f. klin. Med.*, 1890, Suppl., xvii, 77.

[25] Opie, *Jour. of Exper. Med.*, 1907, ix, 207.

ferments from the leucocytes and the lymphocytes, leucoprotease and lymphoprotease, and discovered that these enzymes are held in check by an antibody present in the serum. He was able also to isolate this antibody and to show that in a relatively small quantity it can neutralize the action of the autolytic ferments.

The researches of Opie explain why a mineral solution is not able to preserve tissues in normal condition, since autolysis soon occurs. To hold in check the activity of the proteolytic ferments set free by the suppression of the circulation, it is necessary to use a fluid containing in some proportion the antibody of the serum. The simplest method would be to use normal serum for perfusing the kidney. Another method consists of cooling immediately the organ to $+1°$ C. a temperature at which the enzymotic activity is almost completely suppressed.

In the first experiments, the kidneys were thoroughly perfused with Locke's solution. In the last ones, the perfusion was very incomplete, a great deal of blood being still mixed with Locke's solution. This change was made with a view of leaving in the vessels of the kidney a little normal serum and contained anti-enzymotic bodies. But the amount was probably insufficient to prevent autolysis.

After the circulation has been reëstablished, the kidneys are not, however, in normal condition, and their cells are still exposed to many causes of injury.

The blood pressure of the host may differ from that to which the kidneys were accustomed. Possibly, the serum of the host is injurious, in some cases, to the new organs. Generally, however, the serum of an animal has no cytolytic action on the cells of another animal of the same species. Nevertheless, Ehrlich has shown that isocytolysins exist. Consequently, it may happen that the cells of the transplanted kidneys are injured by the serum of the host, even of the same species. This is probably an exceptional complication.

The isolation of the nervous apparatus of the kidneys from the central nervous system may be the cause, direct or indirect, of anatomical lesions. The denervation appeared as a grave objection to the possible efficiency of the transplanted kidneys. There is no

physiological evidence of the existence of secretory renal nerves. However, denervation of the kidney produces, according to Bindo de Vecchi,[26] degenerative lesions of the epithelial cells of the tubules. Marked disorders of the renal functions followed the section of the nerves in the experiments of Krimer, Brachet, Muller and Peipers. The urine, very abundant and diluted, contained albumin and even blood corpuscles. It is, of course, impossible to ascertain whether these changes are due to the section of the hypothetical secretory nerves or merely of the vaso-motor nerves or, perhaps, to other secondary causes. But, even if the denervation alone be able to bring about these results, it need not be considered as especially dangerous. An animal can live in good health after section of the renal nerves. Floresco[27] dissected and cut out the nerves of the left kidney of a dog. Fifteen days afterwards, he resected the right kidney. The animal remained in good health. Last year, an experiment still more complete and conclusive was performed by me at the Rockefeller Institute. In the same operation, the right kidney of a bitch was extirpated and the left kidney isolated and left united to the body only by its ureter and vessels which were dissected as closely as possible. Practically all the nerves were severed. Nevertheless, five days after the operation, the amount of urine voided was 130 cubic centimeters. There was no albumin and the animal was in a normal condition. After one month the animal was found secreting from 90 cubic centimeters to 124 cubic centimeters of urine with high specific gravity and without albumin in the twenty-four hours. Eight months after the operation, he was in excellent health. This demonstrates that the denervation of the kidneys is of little importance for the general health of the dog. It is probable, however, that the kidneys, being deprived of the powerful protection of their nervous system, are more sensitive to pathological insults than the normal kidneys are. Had the animal been allowed to live as street dogs do instead of being kept quietly in a cage at an even temperature, with good food and without muscular exertion, perhaps pathological changes would have ensued.

[26] de Vecchi, *Arch. di farmacol. sperimentale e scienze affini*, 1906, v, 433, 479.
[27] Floresco, *loc. cit.*

CONCLUSION.

Among so many etiological factors, it is impossible to discriminate which are responsible for the complications which took place in our experiments. An attempt to explain the occurrence of nephritis, œdema or calcification of the arterial system, for instance, will not be made, but the technique of the operations will be modified in order to suppress as much as possible the causes which may originate these secondary changes. The purpose of this article was not to analyze minutely the physiological or pathological character of the functions of transplanted kidneys, but merely to ascertain whether these functions are efficiently reëstablished.

It is to be concluded that an animal which has undergone a double nephrectomy and the grafting of both kidneys from another animal can secrete almost normal urine with his new organs, and live in good health at least for a few weeks. This demonstrates that it is possible to reëstablish efficiently the functions of transplanted kidneys.

EXPLANATION OF PLATES.

Plate XI.

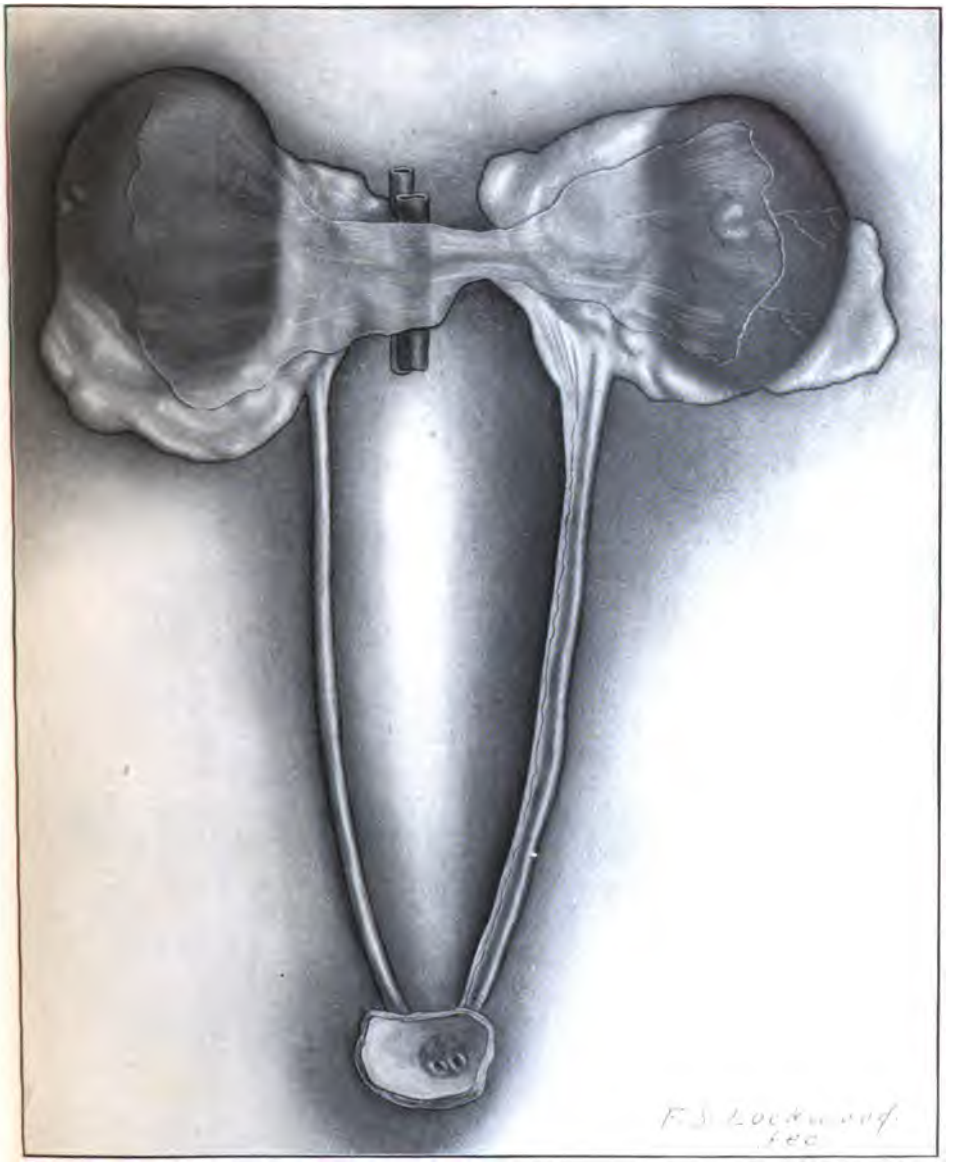
Anatomical specimen extirpated from the first animal and ready for transplantation to the second animal (host).

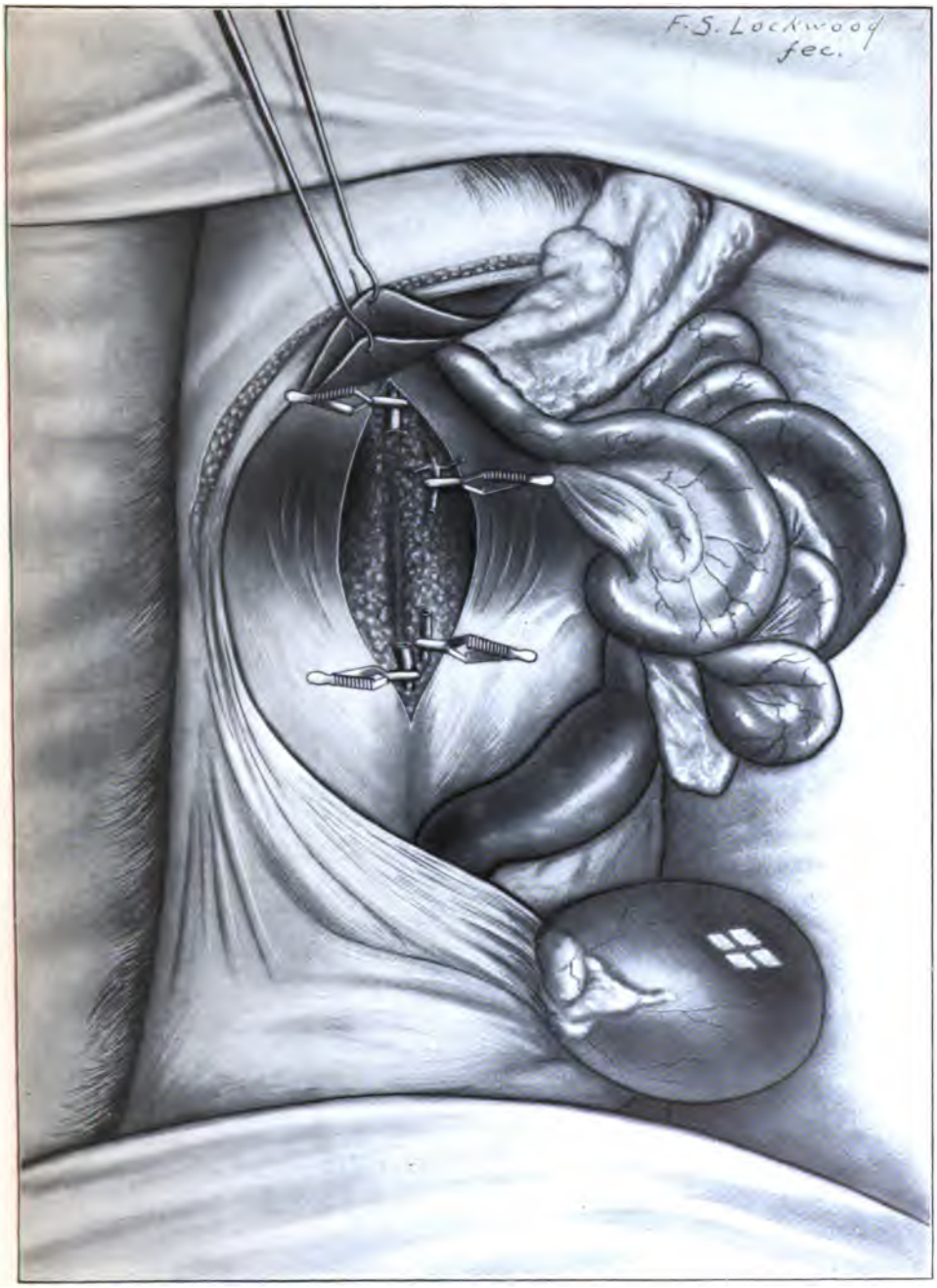
Plate XII.

The host ready for the reception of the anatomical specimen of Plate I.

Plate XIII.

Specimen taken from Cat 7, showing the transplanted kidneys, and cicatrized vascular anastomoses and flap of bladder.

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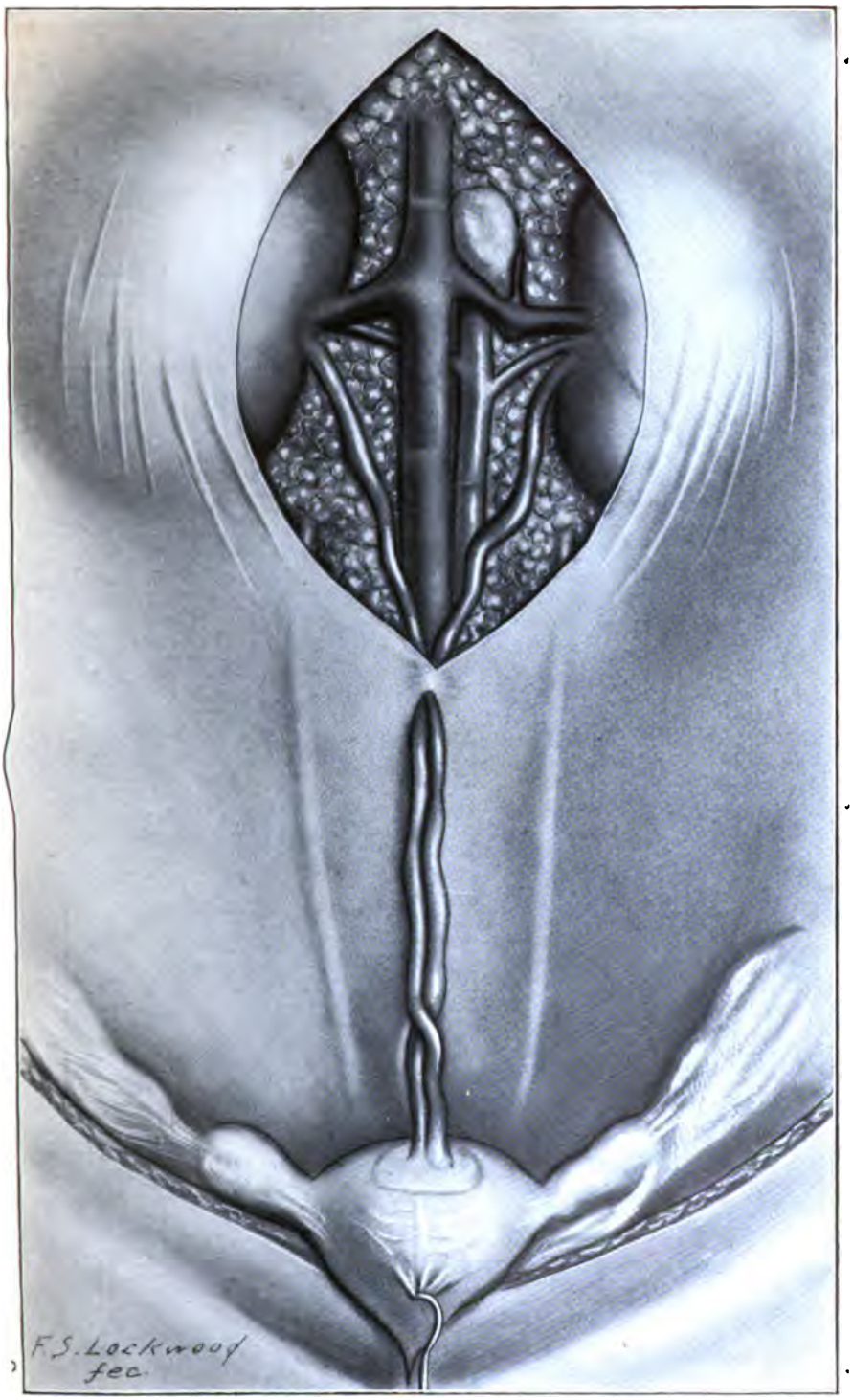
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SERUM TREATMENT OF EPIDEMIC CEREBRO-SPINAL MENINGITIS.*

By SIMON FLEXNER and J. W. JOBLING.

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INTRODUCTION.

During the prevalence of the epidemics of cerebro-spinal meningitis in America and Europe from 1904 to 1907 *Diplococcus intracellularis,* discovered by Weichselbaum in 1887, was established finally as the cause of epidemic meningitis. In the course of the studies of this microorganism carried out by one of us (Flexner[1]), as one of a commission appointed by the Department of Health of the City of New York to investigate epidemic meningitis, an attempt was made to modify favorably the course of experimental infections with the diplococcus in animals by antisera prepared in several kinds of small animals from *Diplococcus intracellularies.* The ultimate purpose of these experiments was the employment of an antidiplococcus serum in the human infection once it was shown that it could be effective in the experimental infections. Flexner's first reports established that guinea pigs and monkeys, in which the conditions of the infection could be controlled, can be saved from otherwise fatal effects of the diplococcus by the use of the antiserum. Up to the time the first report was published a sufficient opportunity to test an antiserum in human beings had not appeared. Since then a diplococcus antiserum prepared by us in the horse has been tested upon several series of cases of epidemic meningitis, occurring in New York, Philadelphia, Cleveland, Castalia and Akron, Ohio, Edinburgh, Scotland, and Belfast, Ireland. The report which follows deals exclusively with the results of the use of the antiserum in human beings affected with epidemic meningitis.

* Received for publication November 9, 1907.

[1] *Jour. of the Amer. Med. Assoc.,* 1906, xlvii, 560. *The Jour. of Exper. Med.,* 1907, ix, 168.

The tests of the antiserum upon which this report rests could not have been carried out without the cordial coöperation of a considerable number of physicians who showed great interest in the undertaking. It will not be possible for us to thank personally, or even by name, all those participating in the tests and we will have, therefore, to content ourselves with the mention of those physicians who were very active in carrying them out. To Dr. L. W. Ladd, of Cleveland, who carried the antiserum to Castalia and Akron, Ohio, and who was the first to employ it systematically in a series of cases of meningitis, we feel an especial and deep obligation, on account of his early interest and the difficulties which he encountered in Castalia in following the cases which arose at widely separated points in a sparsely-settled country district. We are also grateful to Dr. Crile, of Cleveland, who brought to our attention the epidemic at Castalia and selected Dr. Ladd to administer the serum, to the physicians of the City Hospital of Akron, for their interest in the subject and the full reports which they supplied, to Dr. W. T. Longcope of the Pennsylvania Hospital, and Dr. B. F. Royer of the Municipal Hospital, Philadelphia, to Dr. L. Emmett Holt, of the Babies Hospital, and Dr. Strain of St. Vincent's Hospital, New York City, to Dr. Harvey W. Cushing, of Baltimore, Dr. Claude B. Ker, of Edinburgh, and Dr. A. Gardner Robb, of Belfast, and to the attending staffs at the several hospitals who permitted the trials to be made on their patients.

There will now follow the records of the cases of epidemic meningitis treated with the serum which are presented, with a few changes, in the precise form in which they came to us. The only alterations made in the reports consist of abbreviations of the hospital records where certain details could be omitted with a view of saving space and time, and the addition, at the end of each case, of a brief discussion, the purpose of which is to re-present the salient features with especial reference to the influence on the course of the disease exercised by the lumbar punctures and serum injections.

We regret that in some instances we have not yet received the full reports of cases treated with the antiserum, and are, therefore, restricted to the use of brief statements given in letters to one of us

(Flexner). These omissions are at present unavoidable and have been brought about by the great distances from New York at which antiserum is being tried, or by other circumstances which have temporarily caused withholding of the records. We believe that should the epidemic in America suffer a recrudescence the antiserum will receive a larger and more searching test; and, in any case, since the disease is still appearing sporadically over a wide territory in America and threatens to reappear in force in Great Britain, we expect to be able to publish a second and more complete report on the serum treatment of epidemic meningitis at no very distant date.

THE EPIDEMIC OF CEREBRO-SPINAL MENINGITIS AT AKRON, OHIO.

The epidemic at Akron began in April, 1907, and embraced about twenty cases of meningitis. We are greatly indebted to Dr. W. S. Chase for many of the facts on which are based our consideration of this epidemic. Between May 9 and June 16 there were reported to the health officer, as having been treated outside the hospital, nine cases of meningitis of which eight died and one recovered. Dr. Chase states that the patient who recovered presented atypical symptoms and no bacteriological examination of the spinal fluid was made. None of these cases received the antiserum.

Eleven cases of epidemic meningitis, established as such by symptoms and by bacteriological examination, were treated in the City Hospital with the antiserum. Eight of the cases recovered and three died. The period of first injection of the antiserum, the total amount of antiserum injected, and the mode of termination of the disease in the eleven patients are tabulated below.

Case	I,	1st injection	5th day;	total serum injection		82.5 c.c.; recovery by lysis.
"	II.	"	30th hour;	"	"	10.0 c.c.; died.
"	III.	"	12th day;	"	"	20.0 c.c.; recovery by lysis.
"	IV.	"	2d day;	"	"	43.5 c.c.; recovery by lysis.
"	V.	"	7th day;	"	"	25.0 c.c.; recovery by crisis
"	VI.	"	2d day;	"	"	22.5 c.c.; died.
"	VIII.	"	1st day;	"	"	35.0 c.c.; recovery by crisis
"	VII.	"	1st day;	"	"	15.0 c.c.; died
"	IX.	"	2d day;	"	"	105.0 c.c.; recovery by lysis
"	X.	"	1st day;	"	"	25.0 c.c.; recovery by crisis
"	XI.	"	2d day;	"	"	22.5 c.c.; recovery by crisis

Two of the fatal cases were of the fulminating type, and one (Case VI) was injected first with the serum at the end of the second day and again on the fourth day of the illness and died three hours after the second injection. The fulminant cases died six hours and ten hours respectively after the first serum injection.

The total number of cases treated with the antiserum at Akron being eleven it is obvious that little value can be attached to the results stated in percentages. However, the following comparison may be made.

Nine cases of meningitis untreated with the antiserum: Eight or 89 per cent. died and one or 11 per cent. recovered.

Eleven cases treated with the antiserum: Eight or 72 per cent. recovered and three or 27.3 per cent. died.

Eliminating from the calculations the fulminating cases as being beyond reach of treatment, the figures obtained are:

Nine cases treated with the antiserum: Eight or 89 per cent. recovered and one or 11 per cent. died.

City Hospital, Akron, Ohio, Service of Dr. W. S. Chase.

CASE I. V. H. White female, aged 12 years. School girl.

Present Illness.—At 1 a. m. April 28, patient complained of pain in legs and stomach; at 4 a. m. became nauseated and vomited. She remained in bed during the greater part of the day and vomited frequently. Late in afternoon arose and walked out for 15 minutes. At 7 p. m. she became unconscious and voided urine involuntarily. She was restless and noisy until 2 a. m., April 30, when she slept for 2 hours; then the restlessness returned. Rigidity of neck and slight retraction of the head were first noticed on the 30th instant. Admitted to hospital that day.

Physical Examination.—Patient unconscious and restless and screaming, neck rigid, head retracted, pupils equal and react to light, patellar and abdominal reflexes absent, Kernig's sign marked, Babinski not present, herpes on lips. Temperature 99.6°, pulse 100, respiration 24.

May 1. Totally unconscious, restless and noisy; temperature ranged from 99.6° to 102.2°; pulse from 92 to 160.

May 2, 10 a. m. Lumbar puncture, 15 c.c. of opalescent fluid withdrawn. Microscopical examination showed Gram-negative diplococci within and outside of pus cells. Temperature from 99.6° to 102°.

May 3. Condition unchanged.

May 4, 6 a. m. Temperature 101.8°.—8.30 a. m. 10 c.c. opalescent fluid withdrawn by lumbar puncture and *10 c.c. antimeningitis serum injected.*—10 a. m. Temperature 100°.—10.40 a. m. Convulsion involving face, eyes and left hand lasting two minutes.—11.21 a. m. Second convulsion, which continued until relieved with chloroform.—1.50 p. m. Continuous nystagmus of both eyes; twitch-

ing of arms and upper lip; reflexes of extremities and cornea absent.—3 p. m. Convulsions continue except when controlled with chloroform. Kernig's sign more marked than before; rigidity of neck increased; convulsions controlled with chloroform until 5.30 p. m., nystagmus continuous between convulsions.—6 p. m. Temperature 102.2°.—8 p. m. 99.8°.—8.45 p. m. Conscious.—9.30 p. m. Convulsion controlled with chloroform.—12 midnight. Temperature 98.2°.

May 5. Temperature did not rise to 100° until 9 p. m. Patient slept from 12.30 a. m. to 3 a. m. Still some twitching of mouth and left eye. At 4 a. m. took water on being aroused. At 6 a. m. complains of thirst and asks constantly for water and milk.—7 p. m. The patient has taken freely of milk and water during the day and cries for food. She has rested well until midnight. Temperature below 100° until 9 p. m., when it was 100°, and at midnight 101.4°.

May 6. Rested fairly well; condition improved; took considerable nourishment.

May 7 and 8. No essential change.

May 9. Restless after midnight, complains bitterly of frontal headache; temperature rose at 12 m. to 102.8°, and at 3 p. m. to 103.4°.—9 p. m. Temperature 103.8°; lumbar puncture under chloroform anæsthesia, 30 c.c. opalescent fluid withdrawn and *7.5 c.c. antiserum injected.*—10.30 p. m. Temperature 101.8°. —12 midnight. 101.6°.

May 10, 2 a. m. Slight twitching of hands; vomited. Rested well and took nourishment during the day; temperature normal to subnormal.

May 11. Temperature remained below 100°; patient rested well.

May 12, 3 a. m. Temperature 100°; very restless and noisy. Temperature rose during the day.—9 a. m. 102.8°.—12 m. 103.6°.—9 p. m. 104.2°. Withdrew, under chloroform anæsthesia, 60 c.c. opalescent spinal fluid, in which diplococci were not found on microscopical examination, and injected *7.5 c.c. of the antiserum.*—12 midnight. Temperature 102°.

May 13. Patient had a good day and the temperature was 100° at 6 a. m., normal at noon and 97° at midnight.

May 15. Complains of bad headache; temperature rose in afternoon to 104° (3 p. m.).—9 p. m. Temperature 103.2°, lumbar puncture yielding 22 c.c. opalescent fluid free of diplococci.—12 m. Temperature 103.8°.

May 16. Temperature remained above 100° until 9 p. m. Vomited several times; very restless. The condition fluctuated, but remained essentially unchanged until May 25. The rigidity of the neck continued and Kernig's sign was still present and the reflexes had returned in some degree. Pain was still complained of in the head and other parts. The temperature fluctuated between 99° and 102.5°. At 11 a. m. 90 c.c. of less opalescent fluid were withdrawn by lumbar puncture and *20 c.c. of antiserum injected.* The spinal fluid contained leucocytes but no diplococci. At 8 p. m. marked urticaria developed. The patient vomited and was restless and noisy during the afternoon. The temperature fell and reached normal at 12 p. m. the next day. The temperature remained at normal or a little below for four days, then rose to 101.8°, and fell in a few hours. The general condition was better.

June 1. Herpes appearing on lips; neck rigidity lessened; Kernig's sign still marked. Temperature fluctuated between 99.4° and 101°.

June 3. Restless, noisy, complains of pain. Temperature rose suddenly at

9 p. m. to 103°.—11 p. m. Lumbar puncture under chloroform anæsthesia. 75 c.c. of fluid withdrawn and *15 c.c. of antiserum injected.* The spinal fluid showed on microscopical examination a few diplococci and a very small number of leucocytes. Temperature at midnight 100.4°. The temperature was normal June 4, and did not rise above again. The note on June 9 reads: "Patient's condition greatly improved; she is rational and quiet all the time, the reflexes are normal, Kernig's sign is absent, the rigidity of the neck has disappeared, there is complete absence of all pain, and all the functions appear to be normal. The patient states that she feels well."

June 10 to 14. The note states that the temperature has remained practically at normal; sleep is good, but there is some complaint during the day of pain in various parts; the reflexes are normal and Kernig's sign is absent, but there is involuntary movements of bowels and bladder.

June 15. The note states that the patient still complains of pain and is very restless and noisy. The temperature is about normal. 2 p. m. Headache ano vomiting. At 3 p. m. lumbar puncture was performed and 75 c.c. of spinal fluid were withdrawn. Microscopical examination showed "many extracellular diplococci and no leucocytes." *22.5 c.c. of antiserum injected.* The next notes are given entire.

"From June 17 to 25, inclusive, the temperature ranged from normal to 101.6°. The latter temperature was reached on the 18th instant; after that it did not again reach 100°. Appetite was good. Pain in the ear and abdomen complained of responded to palliative treatment. Involuntary micturition and bowel movements continued. On the 25th the patient sat up in bed without discomfort."

"Beginning June 26 (58th day of disease) the temperature remained normal until the day of discharge (96th day of disease). After June 28 the involuntary movements ceased. . . . On 96th day the patient was examined and found well; discharged. She reports to hospital twice weekly, and she is in excellent condition."

Discussion.—This case is one of severe and protracted epidemic meningitis. The diagnosis is clearly established by the symptoms and the bacteriological examination. The special interest which the case has for us is involved in the question whether its course was essentially influenced by the several injections of antimeningitis serum. It does not seem possible to give a definite and outright answer to this question. On the other hand the following points appear to be clear: The first injection of serum (on May 4) was followed by severe convulsions enduring during a large part of the day and requiring to be relieved by chloroform. The convulsions did not re-appear spontaneously and were not excited by subsequent injections of larger amounts of the serum. It is probable, therefore, that the association was accidental. The serum injections generally were followed by a fall in the temperature which reached

or approached the normal and remained at these levels for several days. The temperature and symptoms were subject to much fluctuation, but when the former rose approximately to 104° and the latter became severe, lumbar puncture and serum injection were followed by a tolerably prompt improvement in the patient's condition. On one occasion (May 15), lumbar puncture was performed and no serum injection made and it is noteworthy that the temperature did not fall as in the instance in which the serum injection followed the puncture. The condition of the patient becoming unsatisfactory on May 25 a puncture and serum injection being carried out the temperature fell promptly, remained at or about normal for several days, and the patient's general condition was described as improved. These different results may mean nothing actually, but we put them together since it is desirable to secure light on the independent effects of the puncture alone and the puncture plus the serum injections. Note should be taken of the occurrence of urticaria following the serum injection of May 25. The clearing up of the cerebro-spinal fluid, following the puncture and serum injections, and the disappearance from it of many leucocytes and all *demonstrable* diplococci before the subsidence of the symptoms is shown to be possible. The final history of the case indicates that there may still remain an active focus in the membranes from which a fresh invasion of diplococcus into the spinal fluid may take place, with which is associated a reappearance of certain symptoms and a sudden rise in temperature. Such a relapse would seem to have occurred on June 3 and not to have been attended by a rich outpouring of leucocytes into the cerebro-spinal fluid. If this observation is admitted it is at least worth noting that the condition was quickly controlled by the puncture and serum injection although it is not established that these means were the sole or chief causes of the abrupt termination of the relapse. Only an accurate and painstaking clinical and bacteriological study of protracted and relapsing cases of epidemic meningitis will suffice to determine the manner in which, and the rapidity with which the body's forces unaided deal with the diplococcus. Total amount of antiserum injected, 82.5 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. George Rankin.

CASE II. H. R. White male, aged 17 years. Rubber worker.

Present Illness.—Admitted May 9, 1907, 12 m. The day before admission the patient was at his work, but in the evening he complained of headache and feeling ill, and he went early to bed. At 4 a. m. his family was aroused by his falling out of bed. He was picked up in an unconscious state. He was seen at 9 a. m. by a physician who found him unconscious, restless, throwing himself about, and with a temperature of 104° F. Still unconscious and tossing about on admission to hospital at 12 o'clock noon.

Physical Examination.—Face flushed; perspiring freely; pupils moderately dilated, do not react to light; neck rigid and slight retraction of head; abdominal, patellar and plantar reflexes absent; Kernig's sign present.

May 9, 1 p. m. Temperature 101.6°, pulse 90, respiration 40. Lumbar puncture yielded 60 c.c. of opalescent fluid containing pus cells and extra- and intracellular diplococci.—3 p. m. Temperature 101.6°. Vomited several times during the afternoon; pulse could not be counted during afternoon.—6.30 p. m. Temperature 102.8°.—9 p. m. 103°.—12 midnight. 105.4°, respiration 70. At 11 p. m. lumbar punctured and 60 c.c. opalescent fluid withdrawn and *10 c.c. of antiserum injected.* The microscopical examination of the fluid gave the same results as the first fluid withdrawn. The pulse continued uncountable and the respiration high. Temperature at 3 a. m. 106.2°, at 5 a. m. 107.2°; died at 5.42 a. m. May 10.

Discussion.—This case is an example of the fulminating type of epidemic meningitis. From the appearance of the marked symptoms and the death, less than 36 hours elapsed. At the time of the second lumbar puncture and the first injection of the serum the patient was in a critical condition and survived these operations only six hours. It is highly improbable that the serum could influence favorably so severe an infection as existed in this case, but it would have been proper to have injected it earlier—immediately after the first lumbar puncture had established the diagnosis—and in much larger quantity. The reason that the serum was not injected earlier is found in the fact that it had first to be brought from Cleveland; and that the dose was small is explained by the date of the serum's employment, for at that time it was being used very cautiously since we had not yet learned that it could be injected in much larger amount with impunity into the inflamed spinal canal of human beings. Total amount of antiserum injected, 10 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. W. S. Chase.

CASE III. B. S. White male, aged 19 years. Rubber worker.

Present Illness.—Until May 11, 1907, the patient was well. On that day he felt badly, went to bed early and was said to have had a chill during the night. No further history of illness until May 13, when a physician saw the patient who was feverish, restless and complained of pain in head and neck. That night he became unconscious; admitted to hospital next morning (May 14). He was, at that time, unconscious and irrational and very restless, tossing and talking continuously. "Temperature 99°, pulseless at wrist, respiration 32 and shallow, cyanosed."

Physical Examination.—Unconscious and greatly cyanosed young man. Heart beats 56 per minute. Neck somewhat rigid and painful on being moved. Reflexes diminished.

May 14. Temperature varied from 99° to 101.6°. Under stimulants, pulse improved; at 12 p. m. 104, at 12 a. m. 88.

May 15 and 16. Condition essentially unchanged.

May 16. Reflexes absent from extremities and abdomen; neck rigidity marked; Kernig's sign present. Lumbar puncture unsuccessful. Restless and talking.

May 23. Note states: "Patient semi-conscious part and conscious other part of the time. The reflexes, except the plantar reflex, are absent. Neck rigid and painful on being moved. Temperature fluctuated from 99° to 102.4°. Slept and rested well and took considerable nourishment."

May 24. Temperature rose to 103.4°; otherwise no change.

May 26. No marked change. Kernig's sign present; reflexes absent. Temperature at 12 p. m. 103.6°. Lumbar puncture performed under local anæsthesia and 60 c.c. opalescent fluid obtained and *20 c.c. antiserum injected.* The examination of the spinal fluid showed pus cells and extra- and intracellular diplococci.—3 p. m. Temperature 103.2°.—6 p. m. 102.8°.—9 p. m. 102°.—12 a. m. 99.8°. Patient rested well, perspired freely and took considerable nourishment during the night.

May 27. Temperature ranged from 98° to 99.2°. The note states: "Mental condition improved; reflexes not changed; slept well and took considerable nourishment."

May 28. Temperature ranged from 97° to 97.8°. The note states: "Reflexes are now normal; the mental condition is good, but the neck is still somewhat rigid and slightly painful on being moved."

The next note states that from May 29 to June 6, the date of discharge from the hospital, the patient's condition continued to improve and there was complete recovery.

Discussion.—This case is an example of epidemic meningitis with severe onset and gradual subsidence of symptoms by lysis. The indications are that the patient's recovery was reasonably assured before the successful lumbar puncture and injection of the serum on the twelfth to the fourteenth day of the disease. Until the day of injection the temperature had not remained continuously, during any

one day, below 101°. Within twelve hours of the injection the temperature fell below 100° and did not again rise to that point, tending rather to remain somewhat subnormal. Whether this is more than a coincidence cannot be decided now. The quick return of the reflexes following the puncture and serum injection, and the acceleration of the rate of improvement in the patient's mental and general condition, may also be merely co-incidental, but they were sufficiently great to be regarded as noteworthy. Total amount of antiserum injected, 20 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. L. R. C. Eberhard.

CASE IV. B. K. Female, aged 15 years. Factory worker.

Present Illness.—Patient felt well and worked until May 14, 1907. Awoke 6 a. m. May 15 feeling ill and complaining of headache; ate little breakfast; returned to bed, vomited and became unconscious during the day. Physician called; found temperature 101.4°; patient restless and irritable and crying out with pain on being touched. Admitted to hospital May 16. She was unconscious and tossing about. The abdominal reflex was absent and the patellar and plantar reflexes greatly diminished. The neck was somewhat rigid and attempts to move it were very painful.

May 16, 9 a. m. Temperature 103.8°, pulse 84, respiration 34.—10.30 a. m. Lumbar puncture under chloroform anæsthesia; 45 c.c opalescent fluid containing pus cells and extra- and intracellular diplococci withdrawn. *15 c.c. of antimeningitis serum injected.*—12 p. m. Temperature 102°. It ranged during the rest of the day from 101° to 102.6°.

May 17, 3 a. m. Temperature 99.6°; 10 a. m. 100.8°; 5 p. m. 102.2°; 12 a. m. 102.6°. The note states: "Patient slept part of the night, but is restless at times. Knee, plantar and abdominal reflexes are present. There is marked Kernig's sign and no Babinski reflex; no ankle clonus. Restless and noisy from 9 p. m. to midnight."

May 19, 8 a. m. Unconscious and irrational except when spoken to. Herpes on lips. Internal squint of right and left eyes.—7.30 p. m. Lumbar puncture under chloroform anæsthesia; 45 c.c. of opalescent fluid withdrawn and *5 c.c. antiserum injected.* Slept from 11 p. m. to 5 a. m.

May 21. Condition has remained essentially unchanged. Temperature, 9 a. m., 103.2°.—9.30 a. m. Lumbar puncture: 3.5 c.c. fluid withdrawn and *3.5 c.c. serum injected.* Spinal fluid shows diplococci.—7 p. m. Condition unchanged.

May 22. Patient irrational and complains more than previously on being moved. She seems not to have control of the arms, although she moves hands, fingers and legs.

May 25. Condition has not changed materially. 9.30 a. m. Lumbar puncture under chloroform; 75 c.c. of opalescent fluid removed; *20 c.c. of antiserum injected.* Temperature during the day ranged from 99.6° to 102.8°.

May 26. Temperature ranged from 99.8° to 102°.

May 27. 99° to 102.6°. The note at 6 p. m. states that all reflexes are present, that the left arm cannot be used and the left trapezius is contracted, drawing the

head to the left side. Patient is conscious and rational and rested well. Kernig's sign is present.

May 27. Urticaria has appeared on knees and elbows. The temperature has remained below 100° since 3 a. m.

May 29. Temperature below 100°.

June 1. The temperature has remained below 100° except for one or two brief intervals, when it reached that height. Patient is conscious and rational. All reflexes except the abdominal reflex are present, the neck is less rigid and can be moved voluntarily as can the arms and legs. Kernig's sign still present.

June 5. Temperature has remained below 100° (all temperatures until to-day taken per rectum). Axillary temperature normal.

June 9. Patient improved in every way. Reflexes normal. Kernig's sign absent. Rigidity of neck gone. Has use of all extremities and functions appear to be normal. Urticaria appeared over entire body.

June 10. The final note states that temperature and pulse remained normal and the patient was discharged well on June 27, the forty-first day after admission.

Discussion.—This case is an example of epidemic meningitis with severe onset, moderately prolonged illness, subsidence of symptoms by lysis and complete recovery. The patient was admitted to the hospital about 24 hours after the appearance of the first severe symptoms, and the diagnosis was established by lumbar puncture and a first dose of antiserum administered within the first thirty hours of the disease. No marked or permanent influence on the course of the disease, as far as can be determined, was produced by the puncture and serum, and subsequently three additional injections of serum (following withdrawal of fluid) were made into the spinal canal. On May 25, or approximately the tenth day of illness, an injection of 20 c.c. of serum was given (the two previous injections were of 3.5 c.c. and 5 c.c. respectively). On May 26, the note states that the reflexes, which had previously been absent, had returned, but the same note records the involvement of the trapezius muscle in the rigidity of the neck. May 27 the temperature remained persistently below 100°, and from that date on no rise of temperature above 100° (rectal measurements) was recorded. Urticaria appeared on that day. The condition of the patient improved more or less in the next days and on June 1, the symptoms had considerably abated, voluntary muscular movements had returned and the neck was less stiff. The disappearance of Kernig's sign and the general functional restoration of the body

are noted on June 9 when the patient was regarded as convalescent. It is not possible to assign the specific influence, if any was exerted, of the serum on the progress and final result in this case. Total amount of antiserum injected, 43.5 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. W. S. Chase.

CASE V. F. N. White male, aged 32 years. Laborer.

Present Illness.—The patient had been well, except for coryza, until May 12, when he felt ill and suffered from pain in the muscles and head. May 13 consulted physician who said he had fever and who gave him a purgative and sent him to bed. Did not go to work, felt chilly, vomited, complained of pain in head and neck. Next day no better, but he went to his work and remained until noon. He was obliged to return to bed, where he remained until May 16, when his mental condition becoming alarming he was taken to hospital. Patient became unconscious in the ambulance.

Physical Examination.—Unconscious; neck rigid; reflexes absent; Kernig's sign absent; head slightly retracted; pupils somewhat contracted, react sluggishly to light. Temperature, 4 p. m., 104.2°; 12 a. m. 102.2°. Delirious.

May 17. The note states that the patient was restless, noisy and delirious, and Kernig's sign was now present.—3 p. m. Lumbar puncture attempted, but no fluid was obtained. At 2 p. m., out of bed, delirious, put in restraint.

May 18. Neck very rigid, otherwise condition unchanged.

May 21. The condition fluctuated during the past four days; the temperature ranged from 98.6° to 104.4°, pulse up to 130, respiration rapid and irregular, and there were delirium, noisiness and restlessness. The physical signs have not changed; hiccough has appeared.

May 22, 10 a. m. Under chloroform anæsthesia lumbar puncture was made and 90 c.c. of cloudy fluid, which was under considerable pressure, were removed. At the same time *25 c.c. of antiserum were injected.* The spinal fluid showed on microscopical examination pus cells and intra- and extracellular diplococci.—12 m. Temperature 100.8°; pulse 104.—12 a. m. Temperature 102°; pulse 98.

May 23. The note states that the patient rested fairly well after midnight. At 9 a. m. he was conscious and resting quietly and the mental condition was improved. He was free from pain and the reflexes were unchanged. Temperature: 3 a. m. 101.4°, 12 p. m. 100°, 12 p. m. 98°.

May 24. Rested well part of night and day. Conscious and rational. Knee and plantar reflexes present. Kernig's sign still present. Neck still somewhat rigid and painful on being moved. Temperature normal since midnight.

May 25. Temperature normal. The patient has slept well and taken freely of nourishment.

The next note states that from May 25 to June 5 the temperature and pulse were normal; the patient was up and about the ward since June 2, the reflexes were normal, there was no rigidity and no Kernig's sign.

June 6. Patient awoke at 3.30 a. m. complaining of pain in legs and back. He was unable to move his legs and complained of pain when they were moved. Reflexes could not be elicited, but legs were held rigidly. Pain and tactile sensations normal.

June 7. Extremities less painful, but there is marked tenderness over the large nerve trunks.

June 8. Functions of lower limbs gradually returning.

June 12. The patient is improving, the neuritis is diminishing and the extremities can be used.

June 15. Patient discharged cured.

August 2. Patient has reported to the hospital. He is in perfect health.

Discussion.—The onset of the symptoms of meningitis was in this case gradual and extended over 3 days. The symptoms became severe on May 16 and the patient's condition was bad until May 23 when it suddenly and critically changed for the better. From May 23 on the patient's condition continued to improve, with the exception of the discomfort caused by the neuritis which appeared in the legs on June 6 and quickly subsided, until June 15 when he was discharged "cured" from the hospital. The meningeal and mental symptoms and the symptoms of general intoxication were, in this case, profound. On May 22, or approximately the seventh day of the disease, successful lumbar puncture was made and the antiserum injected. Twenty-four hours later the patient's condition was described as improved and the level of the temperature was lower than before. The condition of the reflexes was, however, unchanged. Within forty-eight hours of the puncture and serum injection the patient had become conscious and rational, the reflexes had in part returned, the neck was less rigid, the temperature reached normal, and the patient was resting quietly, sleeping and taking nourishment. No rise in the temperature again occurred. The patient was evidently convalescent. The critical disappearance of the severe symptoms and the lumbar puncture and injection of serum would seem to bear some relation to each other. There was an abrupt transition from a condition of much seriousness, which had endured unchanged for a week, to one of comparative and actual mildness within forty-eight hours of the puncture and injection of the antiserum. Total amount of antiserum injected, 25 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. W. S. Chase.

CASE VI. Z. H. White female, aged 17 years. Rubber worker.

Present Illness.—The patient was at work until May 18, when she went home on account of malaise and pain in back and limbs. At 2 a. m. the next morning

she complained of severe headache, at 3 a. m. she became unconscious. A physician was called at 7 a. m. (May 19). He said she had fever. During the day she vomited frequently. Axillary temperature, 3 p. m., 104°. Admitted to hospital 6 p. m.

Physical Examination.—Patient admitted in an unconscious state. She is very restless, tosses and rolls her eyes continuously. Knee and plantar reflexes present; abdominal reflex absent; Kernig's sign present.

May 20. Temperature, 9 p. m., 101.2°. Under chloroform anæsthesia 45 c.c. of spinal fluid were withdrawn by lumbar puncture and *15 c.c. antiserum injected.* The fluid showed pus cells and extra- and intracellular diplococci.—12 p. m. Temperature 99.2°.

May 21. Patient conscious and rational; complains of frontal headache. Pupils equal and react; abdominal reflex absent; patellar diminished, plantar present. Kernig's sign marked. Babinski sign absent; ankle clonus absent. Neck rigid and painful on movement. Temperature has risen above 99°.

May 22. Patient's mental condition less good than yesterday. Herpes appearing on lips. Large hyperæmic areas have appeared on abdomen. Temperature during the day has fallen to 99.8°.—10.30 p. m. Under chloroform anæsthesia withdrew 7.5 c.c. of spinal fluid and injected *7.5 c.c. of the antiserum.*—12 a. m. Temperature 99.2°.—12.30 a. m. Patient had been resting fairly well when the nurse's attention was attracted by gasps and before a physician could reach her side she died. No autopsy was permitted.

Discussion.—The symptoms of meningitis came on quickly and were severe in type and lumbar puncture and a serum injection were made about 45 hours after their first appearance. Following the puncture and serum injection the temperature remained constantly below 100° and the patient regained consciousness. A second puncture and serum injection were made about 48 hours after the first. About two and a half hours after these the patient suddenly died. No autopsy was obtained and the immediate cause of death was not established. Total amount of antiserum injected, 22.5 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. A. F. Sippy.

CASE VII. H. S. White male, aged 14. Schoolboy.

Present History.—Patient complained of headache on May 21. The next day he vomited and suffered pain in back of head. Temperature, 11 a. m. 101°; delirious at 5 p. m. Admitted to hospital at 8 p. m.

Physical Examination.—Temperature 104°, pulse 120, respiration 44. Wildly delirious; pupils dilated, do not respond to light. Reflexes absent; Kernig's sign positive; some rigidity of neck.

May 22, 9 p. m. Under chloroform anæsthesia 75 c.c. of opalescent spinal fluid withdrawn and *15 c.c. of antiserum injected.* The spinal fluid contained pus cells and extra- and intracellular diplococci. Temperature, 12 a. m., 103°; pulse 100; respiration 30.

May 23. Patient slept until 2 a. m., after which time he was restless. Temperature: 3 a. m. 102.4°, 9 a. m. 99° 9 p. m. 100° (by rectum).

May 24. Temperature: 12 p. m. 100.6°, 9 p. m. 99.8°. Pulse 60 to 78. Vomited twice; rested fairly well.

May 25. Patient slept fairly well after-part of night; complains of headache. Neck rigid and painful on motion; pupils react to light. Patient conscious and rational. Temperature: 1 a. m. 103°, 9 a. m. 102°. At 10 a. m. 75 c.c. of opalescent spinal fluid withdrawn and *20 c.c. antiserum injected.* Microscopical examination of the spinal fluid showed leucocytes and diplococci. Temperature: 12 p. m. 100°, 6 p. m. 99°, and 12 p. m. 98.6° (mouth).

May 26. Temperature normal. Patient slept well after-part of night; conscious and rational; rests quietly; all reflexes present; neck less rigid than earlier in attack.

May 27 to June 1. The temperature has remained normal and his condition satisfactory. Note on June 2 states that the patient is apparently well, the reflexes are normal and the rigidity of the neck has gone.

The final note states that from June 3 to June 8, the day of discharge from hospital, the temperature remained normal and recovery was perfect.

Discussion.—The patient was brought to the hospital during the first day of illness and the diagnosis of meningitis was established by lumbar puncture and the first dose of serum administered in little more than 24 hours after the illness began. The subsequent course of the infection was mild. On the fourth day of illness (May 25) the temperature having risen to 103° F. a second puncture and serum injection were made. Less than twenty-four hours later the temperature had fallen to normal and the general condition of the patient had improved. There was no subsequent rise in the temperature, all the symptoms rapidly subsided, and recovery was complete. Total amount of antiserum injected, 35 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. W. S. Chase.

Case VIII. R. M. White male, aged 17 years. Electrician with rubber company.

Present Illness.—On May 24 left his work at noon on account of feeling ill. Went home, refused supper, vomited during the evening, and went to bed early on account of headache. Restless during the night. Headache more severe next morning; vomited again, and became unconscious about 10 a. m. Admitted to hospital 1 p. m.

Physical Examination.—Well-developed, muscular man. Pupils slightly contracted; equal; react to light. Neck somewhat rigid and painful on being moved. Knee reflex exaggerated, plantar reflex present, abdominal reflex absent. Kernig's sign present. The patient is very violent and tosses on the bed.

May 25, 2 p. m. Temperature 105.2°, pulse 142, respiration 20. Under chloroform anæsthesia 15 c.c. of spinal fluid withdrawn and *15 c.c. of antiserum injected.* The spinal fluid showed leucocytes and extra- and intracellular diplococci.—6 p. m. Temperature 104.8°, pulse 134.—9 p. m. Temperature 102.4°, pulse 130, respiration 20, cyanosed. Aromatic spirits of ammonia and ether administered hypodermically. Respiration ceased; artificial respiration and oxygen inhalation; respiration continued from 6 to 8 per minute; death at 12.30 a. m.

Discussion.—The preceding case is an example of the fulminating type of epidemic meningitis in which from the first appearance of severe symptoms and death about 24 hours elapsed. Lumbar puncture and serum injection were made about 10 hours before death and were without appreciable effect on the course and termination of the infection. Total amount of antiserum injected, 15 cubic centimeters.

City Hospital, Akron, Ohio. Service of Dr. George Rankin.

CASE IX. J. A. S. White male, aged 24 years. Mail carrier.

Present Illness.—Until two days before admission to hospital patient has been in good health. He ascribed headache and malaise to exposure to hot sun. June 23 had intense headache, nausea, vomiting and pain in back of neck. About 11.30 p. m. became unconscious. Temperature 103°. Admitted to hospital June 24, 11 a. m.

Physical Examination.—Patient violently delirious; requires to be restrained. Had involuntary bowel and bladder movements. Abdominal and patellar reflexes absent; plantar reflex diminished. Neck rigid; painful on being moved. Kernig's sign present. Pupils equal, moderately dilated, react to light.

June 24, 11 a. m. Temperature 103.8°, pulse 70, respiration 36.—1 p. m. Under local anæsthesia 90 c.c. of spinal fluid removed and *22.5 c.c. antiserum injected.* Pus cells and diplococci chiefly within the cells found on examination of the spinal fluid. Temperature: 3 p. m. 100.8°, 12 p. m. 101.4°.

June 25. Patient slept fairly well under an anodyne during the latter part of the night. Note at 8 a. m.: "Patient quiet and semiconscious, responds when spoken to but mutters unintelligibly when attempting to form sentences. Knee jerk not elicited; plantar reflex diminished; abdominal reflex present for first time since admission. Neck rigidly increased." The temperature fluctuated from 99.6° (6 p. m.) to 101.2°.

June 26. Patient restless during night; conscious and rational part of time; neck held less rigidly. The temperature has risen: at 12 p. m. 102.2°, at 12 a. m. 103° (per rectum).

June 27. Restless; extremities cold. 3 a. m. lumbar puncture under chloroform anæsthesia; 45 c.c. of spinal fluid withdrawn and *22.5 c.c. antiserum injected.* A chill followed lasting 20 minutes. Herpes appearing on lips. The temperature has fluctuated between 102° and 103° all the day.—9 a. m. "The patient in exceptionally cheerful mood, singing and talking, but entirely rational when spoken to."

June 28. Condition essentially unchanged.

June 29. Temperature at a slightly lower level.

June 30. The note reads: "Condition good; perfectly conscious and rational; reflexes all present; Kernig's sign still marked; neck quite rigid and painful on movement; headache; extensive herpes labialis." The temperature fluctuated between 101° and 103.2°.

July 1. Condition about the same.

July 2, 6 p. m. Temperature: 103.4°, 9 p. m. 103.2°. Under chloroform anæsthesia 60 c.c. spinal fluid, containing diplococci, withdrawn and *30 c.c. of antiserum injected.*—12 a. m. Temperature 101.8°.

July 3. The temperature reached 99° and has run at a lower level than previously.

July 4. The temperature (rectal) has remained below 100° all day. Otherwise the condition is not markedly changed.

July 5. The temperature has fluctuated to-day, having reached 102.2° at 9 a. m., but remaining below 100° for the most part.

July 6. The temperature has risen again. At 6 a. m. it was 103.8°; patient did not rest well; the neck rigidity has lessened; mental condition good. Kernig's sign still present.—12 p. m. Temperature 103.4°. At 2 p. m. 45 c.c. of spinal fluid were withdrawn and *30 c.c. of antiserum injected.* Stained specimens of the spinal fluid show very few leucocytes containing diplococci and extracellular diplococci.

July 7. Temperature at a lower level, but general condition not essentially changed.

July 8. Patient rested well. The temperature has been below 100° most of the day.

July 9 and 10. Temperature has not risen and condition of patient improved.

July 11 and 12. The temperature has remained normal.

The final note reads: "July 13 to July 27, the date of his discharge from the hospital, the patient continued to improve. When discharged he complained only of not feeling strong. On August 24, when he reported to the hospital, he was well but had not recovered usual strength. On September 8 he had not yet reported for work."

Discussion.—This case of epidemic meningitis ran a moderately severe and protracted course and the symptoms gradually subsided. The particular influence of the spinal punctures and serum injections cannot be defined. The disease terminated favorably and recovery was complete. The conditions under which the serum was employed were favorable to its action since the first injection was made within 48 hours of the onset of severe symptoms. Four injections of the serum were made without the appearance of unpleasant effects. On the other hand the temperature tended to seek a lower level after the injections. The third injection, on the tenth day of illness, was followed by a fall in the temperature

which persisted for four days. When it again rose to 103.4°, on the fourteenth day a fourth injection of the serum was made after which the temperature fell below 100° and soon reached normal where it remained. Convalescence may be said to have begun on the sixteenth day of the illness. Total amount of antiserum injected, 105 cubic centimeters.

City Hospital, Akron, Ohio. Service of Drs. Kobler and Seiler.

CASE X. E. R. White male, aged 20 years.

Present Illness.—The illness began with headache and nausea about noon. After midday meal the patient went to bed and at 3 p. m. was found unconscious and in a violent state. Admitted to hospital the same day (June 30, 1907) at 9.50 p. m.

Physical Examination.—Unconscious and violently excited man moaning constantly. Knee and plantar reflexes exaggerated; Kernig's sign positive; neck slightly rigid; pupils moderately dilated; react to light.

June 30, 10 p. m. Did lumbar puncture under chloroform anæsthesia and withdrew 75 c.c. of spinal fluid and injected *25 c.c. antiserum.* Microscopical examination of the fluid showed polynuclear leucocytes and many intra- and extracellular diplococci. Temperature: 10 p. m. 100.2°, 12 a. m. 99°.

July 1, 2 a. m. Convulsion lasting 20 minutes. During the day patient vomited several times. The temperature did not rise above 100°.

July 2. The temperature has been below 100° all day and much of the time has been normal. The note reads: "Condition of patient markedly improved; he is conscious and rational, all the reflexes are present, Kernig's sign is less marked than it was, the neck is still stiff and painful, headache still persists, and the mental condition is good. Considerable nourishment was taken during the day."

July 3. The temperature has remained below 100° and much of the time was normal. The general condition is as on yesterday.

July 4. Patient rested well; he complains less of pain. Temperature normal.

July 5. The note reads: "Patient slept well; general condition good; reflexes normal; Kernig's sign and neck rigidity absent; mentality normal."

The patient continued to have normal temperature and was discharged on July 11 "cured," twelve days after having entered hospital.

Discussion.—The onset of the symptoms in this case was abrupt and severe. From the appearance of the premonitory headache and malaise to the lumbar puncture and serum injection hardly more than twelve hours had elapsed. Following upon the puncture and the injection of serum the symptoms abated rapidly and the patient may be said to have been over the disease within forty-eight hours of its onset. No reasonable doubt can exist regarding the diagnosis in view of the symptoms present and the results of

the bacteriological examination. Total amount of antiserum injected, 25 cubic centimeters.

City Hospital, Akron, Ohio. Service of Drs. Theiss and Sippy.

CASE XI. G. G. White female, aged 17 years. Schoolgirl.

Present illness.—Twenty-four hours before admission to the hospital she began to complain of malaise and headache. During the night she vomited and the symptoms grew gradually worse until 2.30 p. m. when she became unconscious. Admitted to hospital at 7 p. m. June 9.

Physical Examination.—The patient is unconscious and restless. Abdominal reflex absent; patellar and plantar reflexes are diminished; pupils equal; involuntary bowel movements.

June 10, 9 p. m. Temperature 98.4°, pulse 108, respiration 20. Under chloroform anæsthesia 90 c.c. of opalescent spinal fluid withdrawn and *22.5 c.c. of antiserum injected.* Microscopical examination of the spinal fluid showed many leucocytes and extra- and intracellular diplococci.—12 a. m. Temperature 98.8°, pulse 124, respiration 20.

June 11. Temperature subnormal most of the day. Patient rested fairly well latter part of night. Twitching of face and mouth; head retracted; rational at times.

June 12. Temperature subnormal. Mental condition greatly improved; abdominal and plantar reflexes present; patellar absent. Kernig's sign marked; neck rigid. Herpes appearing on lips. Complains of no pain unless moved.

June 13. Rested well during night. Mental condition quite good; reflexes all present. Kernig's sign and neck rigidity unchanged. In nurse's absence has gotten out of bed and gone to closet. Temperature normal.

June 14. No change.

June 15. Condition good, all the reflexes normal. Almost no rigidity of the neck; Kernig's sign disappearing.

"From this date the patient continued to improve, and she finally made complete recovery. She left the hospital on June 23, thirteen days after admission, and has reported twice since."

Discussion.—The onset in this case was moderately severe, and the diagnosis of epidemic meningitis was established and a serum injection made within forty-eight hours of the appearance of the premonitory symptoms. The course of the disease was relatively mild, and the temperature tended to remain at the normal point or a little below it although the microscopical examination showed the presence in the spinal fluid of large numbers of *Diplococcus intracellularis.* A second lumbar puncture and serum injection were not made as the symptoms abated rapidly and the patient was convalescent on the fifth day of her illness. Total amount of antiserum injected, 22.5 cubic centimeters.

THE EPIDEMIC OF CEREBRO-SPINAL MENINGITIS AT CASTALIA, OHIO.[2]

The village of Castalia has a population of about 600 persons. Within the village nine cases of meningitis developed. In the outlying country and within three miles of the village, six cases developed. In the country adjacent to the village of Vickery, which is eight miles from Castalia, three cases developed. Thus a total of eighteen recognized cases of meningitis developed in this region between January and April, 1907. The first case appeared near Vickery in January and the remaining seventeen cases appeared between March 1 and April 2. Eleven of the eighteen cases were in adults over 16 years of age, and seven cases were in children between three and six years of age. Of the affected adults nine died and two recovered and of the affected children three died and four recovered.

In the past thirty years sporadic cases of the disease have appeared occasionally, but at long intervals. No case had been recognized previously for five years.

The distance between cases No. 1 and No. 2 was six miles; between No. 2 and No. 3, three miles, between No. 3 and No. 4 one mile. The first three cases occurred in the country. Case No. 4 appeared in Castalia and was followed in rapid succession by eight other cases. Only two of the cases had been in close personal relation with other persons affected.

At the time that Dr. L. W. Ladd brought the antimeningitis serum to Castalia there had been twelve deaths from epidemic meningitis and three cases were convalescent. He employed the serum on three cases as follows:[3]

CASE I. B. K. Female, aged 16 years. Patient of Dr. Storey.

Previously healthy girl. Taken ill suddenly March 30, 1907, with headache, vomiting and flushed face. The temperature was 104° F., pulse 124, respirations 48. Coma supervened within 12 hours. The diagnosis was made day after onset, at which time there were opisthotonos and rigidity of the extremities and petechial eruption of neck, trunk and thighs. The patient remained in coma until 12 a. m. March 31, when she became conscious. April 2 again became unconscious. Dr.

[2] Abstracted from the account of Dr. William Storey, published in the *Ohio State Medical Journal* for June, 1907.

[3] The histories of these three cases are taken from Dr. Storey's report and Dr. Ladd's notes.

Ladd first saw the patient at 7 p. m. April 2. At this time she was semi-conscious, the temperature was 103° F., pulse 120, respirations 48. Lumbar puncture was made, about 45 c.c. of very turbid spinal fluid were obtained and *5 c.c. of the antiserum injected.* The spinal fluid yielded *Diplococcus intracellularis* on coverslips and in cultures on sheep-serum glucose agar. April 3. Temperature 98.5°, pulse 108. Patient quite rational at times, though when not aroused she was delirious.—3 p. m. Temperature 101°; 11 p. m. 103.5°. Second lumbar puncture made, 30 c.c. turbid fluid withdrawn and *10 c.c. of antiserum injected.* The patient remained in a semi-conscious condition for 15 hours when the mental condition cleared; the opisthotonos and rigidity were noted to be much less marked. April 4. Acute bronchitis and broncho-pneumonia developed. The mental condition remained good and the meningeal symptoms were not prominent until April 18, when another puncture was made. About 50 c.c. of turbid fluid were removed and *10 c.c. of antiserum injected.* From this time improvement was gradual but steady. April 24, temperature normal; did not rise again. May 3, slight degree of foot-drop on left side. August 31, recovery complete.

Discussion.—The onset in this case was sharp and the course severe. Withdrawal of spinal fluid and injection of antiserum were made on the third day of illness. A favorable response seemed to follow. Two subsequent lumbar punctures and antiserum injections were made on the fourth and fifteenth day of illness. Following the second injection of serum the mental condition cleared and the rigidity of the body was noted as being diminished. The last injection was succeeded by gradual subsidence of all the symptoms and eventual complete recovery. The meningitis was complicated with an intercurrent broncho-pneumonia. Total amount of serum injected, 25 cubic centimeters.

Case II. F. W. Three years of age. Patient of Dr. Gorsuch.

This child was seen by Dr. Ladd twelve days after the beginning of the symptoms. The onset, consisting of vomiting, convulsions, headache, opisthotonos and great irritability, was sudden. Temperature at onset 104°, pulse 135, respirations 40. The patient had improved somewhat, although there was still great irritability and marked opisthotonos; temperature from 101° to 102.5°. April 3, marked opisthotonos and Kernig's sign; temperature 102°. Lumbar puncture yielded 30 c.c. of moderately turbid fluid. *5 c.c. antiserum injected.* That night, for the first time during the illness, the parents were not aroused. April 4, child less irritable; opisthotonos and rigidity less marked; food taken better. The temperature reached normal and remained so afterwards. April 28, child up and about. August 31, child well.

Discussion.—The abrupt change in the condition in this case was evidently associated with the withdrawal of spinal fluid and the

injection of the antiserum, but the part each played cannot be estimated separately. Total amount of serum injected, 5 cubic centimeters.

CASE III. J. B. Aged 23 years. Patient of Dr. Bowman. Employed on railroad. Large man of excellent physique. On April 1 went to Freemont, Ohio, to a hotel. Asked to be called early April 2. The door of his room had to be forced open; the patient was found unconscious. He was removed to his home in Vickery and his physician called. On April 4 Dr. Bowman was convinced of the diagnosis of cerebro-spinal meningitis and sent to Castalia for Dr. Ladd.

Note by Dr. Ladd: Man of powerful build; comatose and restless: thrashing about the bed at times. Marked opisthotonos; purulent conjunctivitis; Kernig's sign present; petechial eruption and larger hæmorrhagic areas on body. Nose-bleed requiring packing to control. Temperature 103.5°, pulse 120, respirations 48 and stertorous. Lumbar puncture; 90 c.c. of turbid fluid removed; *10 c.c. of antiserum injected.* Regarded the condition as hopeless. April 5, in the morning patient still unconscious. Pulse was lower, temperature normal, respirations normal. In afternoon second puncture. 45 c.c. of less turbid fluid removed and *10 c.c. of antiserum injected.* April 6, *10 c.c. of antiserum injected.* Patient still unconscious. April 7, patient coming out of unconscious state. The progress was fluctuating and the patient was not without fever for 30 days. The improvement was gradual and the restoration complete. The patient returned to his work.

Discussion.—The case was one of abrupt and violent onset and of gradual subsidence of symptoms. The first puncture and injection of the serum were made on the fourth day of illness and were followed rapidly by improvement in the temperature, pulse and respirations. Two further injections of serum were given. The recovery was slow and complete. Total amount of serum injected, 30 cubic centimeters.

The epidemic of Castalia embraced 18 cases of meningitis of which 12 died and 6 recovered. Three of the latter recovered without lumbar puncture being performed and without the antiserum being injected. The three cases which were injected with the serum recovered. As these were the first cases in series to be injected with the serum the doses employed were smaller than the ones used subsequently.

The first case was injected with serum about 72 hours after the onset of the disease. Improvement was gradual and the symptoms subsided by lysis, hence it is not possible to assign certainly the

precise value of the serum injections. The second case, which was already in its second week of the disease, seems to offer more certain proof of the value of the puncture and serum injection. The disease terminated abruptly after the injection of 5 cubic centimeters of the serum and recovery was rapid and complete.

The third case was regarded as hopeless. Lumbar puncture and injection of antiserum having been made, the patient's condition changed quickly for the better, but the final recovery was fluctuating and gradual. The precise value of the serum injections must therefore remain doubtful.

EMPLOYMENT OF THE SERUM BY DR. L. W. LADD, OF CLEVELAND.

Dr. Ladd treated in all sixteen cases of epidemic meningitis in which the diplococcus was found with the antiserum in Castalia and Cleveland. He has kindly supplied us with the notes of his cases which will be presented in this place. Since three of the cases treated by Dr. Ladd were at Castalia the histories relating to them are given under the local epidemic of which they formed a part. We will first present Dr. Ladd's own analysis of the sixteen cases, then an analysis of our own, after which the case-histories of each to which we have added a brief discussion will be given.

"The sixteen cases consisted of 11 males and 5 females. Five patients were over 16 years, eight under 5 years and three between 5 and 8 years of age. When first seen thirteen of the sixteen cases were in coma, one was semi-comatose and two were conscious. Five patients were seen 24 hours or slightly earlier after the onset of symptoms. All these cases recovered completely. One patient was seen 48 hours after the onset. The condition was desperate. The patient died. Four patients were seen approximately 72 hours after the onset. They recovered. Two of these cases recovered completely, one developed foot-drop and recovered subsequently, one had impaired hearing when last seen. Three patients were seen approximately 96 hours after the onset. They died. Two of these cases showed a temporary improvement following lumbar puncture and serum injections. Two patients were seen two weeks after the onset, one recovered, the other died of chronic hydro-

cephalus. One patient was seen one month after onset and died of chronic hydrocephalus."

The percentage of recoveries among the sixteen cases was 68.75 and the deaths 31.75. Taking the ten cases treated with serum within 72 hours of the onset of symptoms, nine recovered and one died. The percentage of recoveries in this series is 90. Two of the five cases ending fatally were injected with the serum 96 hours or thereabouts after the onset and two cases several weeks after the beginning of the disease.

Case	I,	1st	injection on	3d	day;	total serum injection	25 c.c.;	recovery by	lysis.
"	II,	"	" 12th	"	"	"	5 c.c.;	"	crisis.
"	III,	"	" 4th	"	"	"	30 c.c.;	"	lysis.
"	IV,	"	" 5th	"	"	"	15 c.c.; died.		
"	V,	"	" 2d	"	"	"	35 c.c.; recovery by crisis.		
"	VI,	"	" 1st	"	"	"	15 c.c.;	"	"
"	VII,	"	" 3d	"	"	"	20 c.c.;	"	"
"	VIII,	"	" 3d	"	"	"	45 c.c.;	"	lysis.
"	IX,	"	" 2d	"	"	"	35 c.c.; died.		
"	X,	"	" 1st	"	"	"	15 c.c.; recovery by crisis.		
"	XI,	"	" 20th	"	"	"	25 c.c.; died.		
"	XII,	"	" 4th	"	"	"	46 c.c.; "		
"	XIII,	"	" 1st	"	"	"	45 c.c.; recovery by crisis.		
"	XIV,	"	" 14th	"	"	"	28 c.c.;	"	lysis.
"	XV,	"	" 3d	"	"	"	53 c.c.;	"	"
"	XVI,	"	" 4th	"	"	"	41 c.c.; died.		

Scrutinizing this table closely we find that of the 11 patients who recovered the disease terminated, after serum injection, by lysis in five and by crisis in six cases. It is somewhat noteworthy to find that a case of meningitis which has lasted twelve days without intermission of symptoms should terminate abruptly after a lumbar puncture and serum injection. But of the several important cases terminating abruptly by crises where the serum injections were made on the first to third day, the most significant is case XIII in which the injection was made about two hours after the first appearance of the meningeal symptoms with the result of immediately arresting the progress of the disease.

A second tabulation dealing with the cases in children under five years of age, owing to the high mortality of epidemic meningitis in infants will be given.

Case	II, Age	3 years;	recovered;	1st injection	on 4th	day of illness.	
"	V, "	3 "	"	"	2d	"	"
"	VI, "	2 "	"	"	3d	"	"
"	X, "	2 "	"	"	1st	"	"
"	XI, "	1½ "	died	"	20th	"	"
"	XII, "	2 "	"	"	4th	"	"
"	XIV, "	1½ "	recovered	"	14th	"	"
"	XVI, "	under 5 "	died	"	4th	"	"

Of the eight cases of this tabulation five recovered and three died. The three deaths occurred in children who received the serum for the first time on the twentieth and the fourth day of the illness respectively. On the other hand two children who were first injected on the fourth day, and one child injected on the fourteenth day recovered. There is no proof that the course of the disease in the last case was essentially influenced by the antiserum.

CASE I. B. K. Castalia epidemic. Recovered.
CASE II. F. W. Castalia epidemic. Recovered.
CASE III. J. B. Castalia epidemic. Recovered.
CASE IV. H. White male, aged 17 years. Barberton, Ohio.

The patient was employed at the Goodrich Rubber Co.'s factory at Akron. May 2, in the evening, he complained of fever and headache. He was seen by Dr. Lahmers May 3. At that time there were diarrhœa and vomiting, but no fever. The same evening meningeal symptoms and coma came on. May 6 Dr. Ladd saw the patient. There were extreme opisthotonos—the head almost touched the scapulæ—general rigidity, Kernig's sign, petechiæ over body, and purulent conjunctivitis. Temperature 103.5°, pulse 85, respirations 30. Lumbar puncture yielded two or three drops of thick pus. *15 c.c. of antiserum were injected.* No beneficial effect was noted. Died May 7.

Discussion.—The character of the exudate in this case and the general condition of the patient five days after the beginning of the infection, probably operated against any beneficial effects resulting from the serum. Total amount of serum injected, 15 cubic centimeters.

CASE V. W. F. Male, aged 3 years. Bohemian.

On March 31 complained of headache in afternoon. April 1, severe headache; vomited. From 1 until 5 p. m. five convulsions, each lasting five minutes.—5 p. m. Coma, opisthotonos and Kernig's sign present; abdominal reflex absent; small petechiæ over body.—9 p. m. Lumbar puncture: 30 c.c. turbid spinal fluid removed and *10 c.c. of antiserum injected.* Temperature 99.4°, pulse 125, respirations 30.

April 2. Patient improved. Temperature and respiration unchanged, opisthotonos less marked, abdominal reflex present. Child conscious and rational; took nourishment.

April 6. Until to-day the child has done well. Temperature suddenly rose to 103.5°, pulse 116.

April 7. Lumbar puncture done in morning and 30 c.c. turbid fluid removed and *15 c.c. of serum injected.* The child was delirious before the puncture.

April 8. Temperature 99.6°, pulse 120.

April 22. Child did well until to-day, when the temperature rose to 103.2°; pulse 132. Vomiting and meningeal symptoms present. Lumbar puncture removed 30 c.c. of fluid; *10 c.c. of antiserum injected.*

April 24. Temperature normal. From this date on the recovery was uneventful and finally was complete.

Discussion.—The first lumbar puncture and serum injection were made within 48 hours of the onset of symptoms. The disease seemed to have been promptly arrested by the injection. Subsequently two relapses occurred, one on the sixth and the other on the twenty-second day, which were as abruptly arrested as the first symptoms by the lumbar puncture and serum injection. Recovery was complete. Total amounts of serum injected, 35 cubic centimeters.

CASE VI. Baby N. Female, aged 2 years. Italian.

Child lived in the poor district of Cleveland. Dr. Steuer, the attending physician, diagnosed the case as meningitis and called Dr. Ladd—22 hours after the onset of symptoms. Child had marked opisthotonos, lateral nystagmus, Kernig's and MacEwen's signs and muscular rigidity. She was completely comatose. Temperature 102.5°, pulse 140, respirations 40. Lumbar puncture: 50 c.c. of turbid fluid removed and *15 c.c. of antiserum injected.* Morning of next day, temperature, pulse and respirations normal; child dull and listless but conscious. Kernig's sign and opisthotonos less marked. Parents refused second puncture and injection of serum. The meningeal symptoms quickly subsided and recovery was complete. A few days later whooping cough developed, from which recovery was finally made.

Discussion.—The essential facts in this case are the sudden onset of severe symptoms of meningitis in an infant of two years and their abrupt arrest and permanent and rapid dissipation after lumbar puncture and serum injection performed in the first first twenty-four hours of the disease. Total amount of serum injected, 15 cubic centimeters.

CASE VII. X. Female, aged 11 years. American.

May 1. Patient seized with severe headache, vomiting, stiffness of neck. 12 hours later unconscious. Opisthotonos marked; Kernig's sign present; lateral nystagmus; petechial eruption. May 4 Dr. Ladd saw patient and did lumbar

puncture. 30 c.c. of turbid fluid removed and *10 c.c. of antiserum injected* (this within 72 hours of the onset of the symptoms). Temperature 103.6°.

May 2. Temperature and pulse normal; patient answered questions rationally though lapsed into semi-consciousness when left alone.

May 6. Improved.

May 7. Temperature rose and meningeal symptoms became prominent. Lumbar puncture repeated; *10 c.c. antiserum injected.* Temperature fell to normal. Complete recovery.

Discussion.—The prompt amelioration of the severe symptoms by the first lumbar puncture and serum injection and the equally prompt suppression by these means of what threatened to be a relapse of the disease, are striking incidents of this case. Total amount of serum injected, 20 cubic centimeters.

CASE VIII. W. H. White male, aged 21 years. Hudson, Ohio. Farmer.

May 12. Had a chill in evening. May 13. Unable to work.—8 a. m. Had a second chill and severe headache. In a few minutes was delirious and soon became unconscious. Dr. Ladd saw the patient May 14. There were present marked opisthotonos, Kernig's sign, general muscular rigidity and petechial eruption. (It developed that two weeks before the patient's mother had nursed a fatal case of epidemic meningitis.) Lumbar puncture was attempted four times but only a few drops of bloody serum were obtained. This fluid showed doubtful diplococci. The culture was negative. Clinically there was no doubt of the diagnosis. In view of the fact that no spinal fluid was obtained, *5 c.c. of antiserum* were injected into the canal and *10 c.c. antiserum under the skin.* Temperature 103.5°.

May 15. Patient improved; rational at times, opisthotonos, muscular rigidity and Kernig's sign less marked. *15 c.c. antiserum injected subcutaneously.* The temperature ranged from 100° to 103°, when, on the twelfth day, it fell to normal and remained there. On the seventh day of illness another dose of *15 c.c. of the antiserum* was given subcutaneously. A marked erythematous rash appeared on the fifteenth day. Recovery was complete.

Discussion.—The absence of bacteriological confirmation of the diagnosis in this case is an evident weakness, but there would appear to be little doubt of the nature of the disease. What is noteworthy is the subcutaneous employment of the antiserum. The symptoms gradually subsided by lysis and hence the special influence of the serum cannot be defined with certainty. Total amount of serum injected, 45 cubic centimeters.

CASE IX. C. G. White female, aged 6 years.

The patient was one of three children in one family afflicted with meningitis. June 30, at 4 a. m., was taken with headache and vomiting. July 1, at 10 a. m.,

had a convulsion. She was brought comatose to the City Hospital. There were marked Kernig's sign and opisthotonos. Extensive petechiæ and herpes oris. July 2, 8 p. m., lumbar puncture yielded 5 c.c. very turbid fluid. *10 c.c. of antiserum were injected.*

July 3, 10.30 a. m. Lumbar puncture done and 15 c.c. very turbid fluid removed. *10 c.c. antiserum injected intraspinally and 5 c.c. subcutaneously.*

July 4. Right-sided hemiplegia developed. Patient's condition still very bad.

July 5, 11 a. m. Lumbar puncture and administration of *10 c.c. antiserum.*—7 p. m. Condition very bad. Temperature 107.4°. Death. Consciousness never regained.

Discussion.—The onset of symptoms was in this case rapid and severe. The first lumbar puncture and serum injection were made about 64 hours after the first symptoms were noted, and while the patient was in an unconscious state. Two later injections of the serum were given, but no beneficial effect followed any of the injections. Total amount of serum injected, 35 cubic centimeters.

CASE X. J. C. White male, aged 2 years.

This is the second child having meningitis in this family. July 1, patient put to bed in good health. Later in the evening he was found restless and feverish. The physician made a diagnosis of meningitis. Admitted to City Hospital July 2, 9 a. m.

July 2. On admission marked opisthotonos, Kernig's sign and general petechial eruption were noted. Child comatose. At 4 p. m., approximately 20 hours after onset of symptoms, lumbar puncture performed and 50 c.c. of turbid fluid removed; *10 c.c. of antiserum injected into spinal canal and 5 c.c. under the skin.* Temperature at this time was 104°, pulse 140, respirations 62.—7 p. m. Temperature 105.6°, pulse 148, respirations 78.

July 3. This morning temperature 100°, pulse 128, respirations 40. Child conscious and irritable.

July 4, 10.30 a. m. Lumbar puncture negative (probably unsuccessful). The patient's condition quickly improved and, except for a slight rise of temperature to 102.4° at 12 a. m. July 5, the temperature, pulse and respirations remained normal.

July 17. No complications have developed. Child discharged cured.

Discussion.—The onset of symptoms in this case was sudden and severe. Twenty hours after the symptoms appeared lumbar puncture and serum injection were performed. Within twenty-four hours of these operations the condition had changed abruptly for the better and the disease had terminated by crisis. Recovery was uninterrupted. Total amount of serum injected, 15 cubic centimeters.

CASE XI. P. G. White male, aged 18 months. Austrian.

March 12. Admitted to Lakeside Hospital. Until March 4 the child had been well since birth. On that day was fretful and feverish, coughed and had diarrhœa. March 7, right elbow swollen and painful; neck stiff; macular eruption over body. On admission to Hospital there were irritability, large head retracted to right side, slight strabismus, pupils equal and active to light, slight swelling about flexed and rigid right elbow.

March 23. Temperature 105°. Lumbar puncture: 25 c.c. slightly turbid spinal fluid removed. On microscopical examination a small number of leucocytes containing diplococci of typical intracellularis appearance seen. No change in condition as result of puncture. Lumbar puncture every third or fourth day as routine. Temperature ranged from 98° (a. m.) to 104° (p. m.). No marked change in the physical condition.

April 12. Lumbar puncture and *intraspinal injection of 5 c.c. serum.*

April 14. Temperature did not rise above 100.5°, which was the lowest maximum temperature since admittance.

April 16, a. m. Temperature normal. *7 c.c. antiserum injected.* Temperature normal until April 23, when it rose suddenly to 104°, pulse 140, respiration 40. Symptoms of chronic hydrocephalus developed. Several additional punctures and injections of serum were made without beneficial effect. Death on July 30.

Discussion.—The first lumbar puncture was made on the twentieth day and the first serum injection was given on approximately the fortieth day of illness. Following the latter the symptoms diminished and there seemed decided improvement lasting eleven days. The symptoms reappeared and death resulted from chronic hydrocephalus. Total amount of serum injected, more than 25 cubic centimeters.

CASE XII. F. P. White male, aged 3 years.

Onset sudden on May 18 with headache, vomiting and temperature to 106.5°. Bright red pin-point rash over body. May 20, first convulsion noticed. Opisthotonos pronounced.

May 22 (about 96 hours after onset). Lumbar puncture and 45 c.c. of very turbid fluid withdrawn. Two hours later second puncture, 20 c.c. fluid removed and *15 c.c. antiserum injected.* Temperature 103°.

May 23. Patient brought to Lakeside Hospital. Temperature 101°, pulse 140, respirations 40. Conscious, very irritable, opisthotonos and Kernig's sign moderately marked (as on 22d, when seen by Dr. Ladd). Petechial eruption present.—4 p. m. Lumbar puncture: 50 c.c. turbid fluid withdrawn and *15 c.c. antiserum injected.*—12 a. m. Temperature 101.6°.

May 24. Patient drowsy; neck rigidity increased. 8 a. m. Temperature 102°. —3 p. m. Lumbar puncture: 40 c.c. turbid fluid removed and *16 c.c. serum injected.* Temperature 103.5°, pulse 135.—4 p. m. Chill, marked cyanosis, pulse feeble, respirations poor.—4 p. m. Temperature 106°. After tub bath temperature 104°.—9 p. m. Chill, cyanosis, temperature 106.5°. Death at 1.25 a. m., May 25.

Discussion.—The first lumbar puncture and serum injection were made 96 hours after the onset of symptoms, and on the next two successive days the punctures and injections were repeated. The disease progressed rapidly and continuously and ended fatally approximately six days after the onset. The question arises whether the sudden change for worse on May 24 bore any relation to the puncture and injection of serum one hour previously. There are no data at hand to use in answering this question. In other cases in which daily injections of serum were made severe symptoms did not appear. Total amount of serum injected, 46 cubic centimeters.

CASE XIII. O. C. White female, aged 7 years. Sister of F. C., Case XII.

Until May 21 child well. Symptoms began with fever (to 106°), severe headache, thirst and erythematous eruption over arms and legs. May 22, 2 p. m., child seen by Dr. Ladd.

May 22. Child conscious. No Kernig's sign. No opisthotonos, rigidity of neck or extremities. A bright erythematous eruption over chest and extremities. During the two hours which were employed in the examination of the fluid withdrawn from brother, marked meningeal symptoms developed, convulsions and opisthotonos and coma set in. Lumbar puncture was done, 30 c.c. of turbid fluid were withdrawn, and *15 c.c. of antiserum injected intraspinally.* The patient spent a quiet night and was brought to the Lakeside Hospital May 23.

May 23, a. m. Temperature 100°, pulse 120, respirations 35. Considerable restlessness. Moderate opisthotonos and rigidity of extremities. Unconscious. Kernig's sign present.—4 p. m. Lumbar puncture: 25 c.c. serum under moderate pressure removed and *15 c.c. serum injected.*

May 24. Condition greatly improved. Opisthotonos almost gone. Kernig's sign diminished, child less irritable and conscious though dull. Temperature normal, except for rise to 101.5° following puncture and injection of *15 c.c. of antiserum.* Only a few drops of turbid fluid obtained.

May 25. Temperature normal; mind clear; meningeal symptoms absent. Recovery uninterrupted. The disease in this case terminated in four days from its beginning.

Discussion.—The symptoms in this case set in abruptly and with much intensity. Within the first 24 hours of illness the diagnosis was established by lumbar puncture and the first serum injection made. What is especially important in this case is the fact that the puncture and serum injection were made in less than two hours after the appearance of symptoms due to meningeal irritation. The disease appears to have been arrested by the first puncture and

injection and, although two subsequent injections of serum were made, the symptoms disappeared rapidly after the first injection and the patient was well on the fourth day from the onset of the illness. Total amount of serum injected, 45 cubic centimeters.

CASE XIV. L. S. White male, aged 1 year and 7 months. Pole.

May 25. Admitted to Lakeside Hospital. Two weeks before the child fell while playing and lay for a few moments with head retracted. In a little while fell again in convulsion. On undressing him the mother noticed an eruption on the body. During the next five days the child had four convulsions; has been in bed since with head retracted and squint. On admission to hospital there were noted emaciation, marked opisthotonos and internal squint. Kernig's sign present. Lumbar puncture gave 25 c.c. turbid fluid. Temperature 101°, pulse 165, respirations 45. *5 c.c. antiserum injected.* Temperature normal during next four days.

May 28. Temperature 101.5°. Lumbar puncture gave 15 c.c. clearer fluid. *6 c.c. antiserum injected.* From the first puncture and injection the opisthotonos diminished and the mental condition brightened. Until June 1 temperature normal.

June 1, 12 a. m. Temperature 103.5°; next morning normal. Child gaining steadily in weight.

June 12. Temperature 103°. Lumbar puncture yielded 25 c.c. of clear spinal fluid in which a small number of diplococci were still present. *7 c.c. antiserum injected.* Until June 25 steady improvement.

June 25. Temperature 102°. *5 c.c. of serum given under the skin.* Temperature returned to normal but on June 27 again rose to 102°. *5 c.c. serum given subcutaneously.* No further rise occurred. Discharged cured.

Discussion.—This case is an example of sub-acute epidemic meningitis in which the symptoms rapidly ameliorated after lumbar punctures and serum injections began two weeks after onset of the disease. The only point that is especially noteworthy is the rapid clearing of the spinal fluid after the first puncture and serum injection. No statement of the precise part played by the serum can be made. Total amount of serum injected, 28 cubic centimeters.

CASE XV. C. D. White male, aged 8 years. German.

The patient was well until the night of June 13. Onset of disease was with irritability, severe headache and vomiting. June 16, diagnosis of meningitis made. There were present marked opisthotonos, muscular rigidity of extremities and petechial eruption. Patient comatose, respirations stertorous.

June 16, 1 p. m. Temperature 103°. Lumbar puncture gave 45 c.c. of turbid fluid; *10 c.c. antiserum injected.*—8 p. m. 45 c.c. spinal fluid removed and *5 c.c. antiserum injected.* The symptoms ameliorated somewhat during the day.

June 17. Temperature and pulse normal. Toward evening patient can be made to respond to loud questions but answers are unintelligible.

June 18, a. m. Temperature 104°; restless.—9.50 p. m. Lumbar puncture: 40 c.c. of turbid fluid withdrawn and *15 c.c. of antiserum injected.*

June 19, 12 a. m. Patient quiet, temperature normal. Mental condition clearer, temperature not above 101.5° for two days.

June 21. Restlessness; temperature 104°. Lumbar puncture: no fluid obtained, *5 c.c. antiserum injected under the skin.*

June 22. Temperature normal.

June 25. *5 c.c. serum subcutaneously.*

June 26, 12 a. m. Temperature 103°.

June 27, 8 a. m. *5 c.c. serum subcutaneously.* Temperature remained below 102° until June 30, when it rose suddenly to 104°.

June 30 to July 8. Up and down temperature.

July 8. Owing to increased irritability and symptoms of meningeal irritation lumbar puncture made and 35 c.c. of nearly clear fluid withdrawn.

July 10. After two days of nearly normal temperature there was a rise to 105.8°. Lumbar puncture made and *8 c.c. antiserum injected.*

July 11. Temperature normal. No further rise from this time. Patient discharged on July 29 well except for slight impairment of hearing.

Discussion.—The first puncture and serum injection were made on the third day of the disease and were followed by improvement in the patient's condition. The course of the illness was fluctuating and the symptoms subsided gradually and with occasional exacerbations. The lumbar punctures and serum injections, made during the access of the symptoms, appeared to control the fever and reduce the restlessness, while the subcutaneous injections of the serum would appear to have been less effective in this respect. Recovery was incomplete as impairment in hearing remained. Total amount of serum injected, 53 cubic centimeters.

Case XVI. I. L. White, aged under 5 years. Hebrew.

June 18. Until to-day well. Complains of headache. June 19. Severe headache, vomiting, opisthotonos, petechial spots on body. In the evening unconscious. June 22. Admitted to Hospital (approximately 96 hours after onset). Symptoms unchanged except for exophthalmos on the right side and a lateral nystagmus. Lumbar puncture yielded 30 c.c. of very turbid fluid. *6 c.c. of antiserum injected.* Temperature fell from 104° to 100° in four hours; muscles less rigid; mind clearer.

June 23, a. m. Spinal puncture: 30 c.c. fluid removed and *5 c.c. serum injected.*

June 24, 4 p. m. Spinal puncture: 35 c.c. fluid removed and *5 c.c. serum injected.*

June 25. *5 c.c. serum given subcutaneously.* Following this the temperature which had ranged from 104.5° to 100° fell to normal.

June 27, 8 a. m. Temperature rose to 103°. Lumbar puncture, 2.5 c.c. reddish serum obtained and *5 c.c. serum injected.* Until July 9 child seemed to improve. The opisthotonos had diminished; temperature slightly elevated, sometimes reaching 104°.

July 9. Lumbar puncture: 45 c.c. of fluid removed and *5 c.c. of antiserum injected.*

July 13. 37 c.c. fluid withdrawn by puncture and *10 c.c. serum injected.* The temperature remained fluctuant; symptoms of increasing hydrocephalus developed and punctures and serum injections were repeated, but death took place on July 31, 1907.

Discussion.—This child was first injected with serum on the fourth day of the illness after which the acute symptoms ameliorated somewhat. The progress of the disease was not arrested by the several punctures and serum injections and chronic hydrocephalus and death resulted. Total amount of serum injected exceeded 41 cubic centimeters.

THE CASES OF EPIDEMIC MENINGITIS AT PHILADELPHIA.

The cases of meningitis at Philadelphia followed on the heels of the large epidemic at New York and probably constituted part of that epidemic. The report of the use of the antiserum at the Pennsylvania Hospital, supplied by Dr. Longcope, embraces five cases of the disease. Of these, four recovered and one died. We will tabulate these cases according to the period of the first injection of the serum, the total amount of antiserum employed in each case, and the mode of termination of the disease.

Case	I,	1st	injection	on 4th	day;	total serum	injection	15 c.c.;	recovery by crisis.
"	II,	"	"	3d	"	"	"	25 c.c.;	" lysis.
"	III,	"	"	4th	"	"	"	45 c.c.;	" crisis.
"	IV,	"	"	11th	"	"	"	25 c.c.; death.	
"	V,	"	"	10th	"	"	"	15 c.c.; recovery by lysis.	

Pennsylvania Hospital, Philadelphia. Service of Dr. M. J. Lewis.

CASE I. J. J. Negro male, aged 23 years. Stevedore.

Present Illness.—On the morning of July 2, 1907, the patient experienced headache, indigestion, weakness and exhaustion and pain in the back. During the afternoon the headache increased in severity, and a sense of malaise and illness was felt. He was brought to the receiving ward by the patrol service and remained over night. The next morning, as he felt better, he left the hospital and went to the wharf and lay down. Soon afterwards he became nauseated and vomited freely. The pain in the head returned with increased severity and there were pain and stiffness of the back of the neck, chilly and feverish sensations and

general pain of the body. That night (July 3) the patient went home and to bed and in spite of being very ill he went to work the next day. Later he was found unconscious on the wharf and was brought to the hospital (July 4). The note on admission states that the patient is unconscious, the neck is rigid, the urine is voided involuntarily, the temperature is 101° F., the pulse 80, and the respirations 26.

Physical Examination.—Large, robust negro lying unconscious but restless; irrational and somewhat noisy. Skin hot and dry; no rash; pupils react equally to light; slight degree of nystagmus and strabismus; conjunctivæ injected; tongue moist and heavily coated; breath offensive; moderate degree of rigidity; Kernig's sign present; patellar reflexes practically absent; sensations appear to be normal. (Nothing abnormal was discovered in heart, lungs and abdomen.) The urine showed a faint trace of albumen and a small number of hyaline and granular casts and leucocytes, but no sugar.

July 5. The alterations since yesterday are increased rigidity of neck and more marked Kernig's sign. Leucocyte count 15,800.—1 p. m. Lumbar puncture: 20 c.c. of very turbid fluid showing many *Diplococcus intracellularis* and pus cells obtained.—5 p. m. Second puncture: 65 c.c. fluid evacuated and *15 c.c. antimeningitis serum injected.* Temperature 100° F.—6 p. m. Temperature 102.2°, respirations increased, some degree of relaxation, patient quieter and less delirious apparently.—6.30 p. m. Temperature 101°, pulse 96, respirations 32.—9 p. m. Temperature 99.3°, pulse and respiration improved, patient more relaxed and has been perspiring, pupils react to light.—10.40 p. m. Partly rational and can be aroused sufficiently to give name, address and occupation; relaxation still more marked; headache is complained of.

July 6. Condition improved. Temperature 98.3°, pulse 60, respiration 20. Neck slightly rigid only, Kernig's sign greatly diminished; pupils equal and react; conjunctivæ clear; nystagmus and strabismus diminished; patient quiet and rational but is drowsy and somewhat stuporous. The next note reads: "This evening the temperature has gone up again; otherwise the general condition is much improved."

July 7. Temperature normal; condition improved; symptoms abating; headache, strabismus and muscular rigidity less marked; some diplopia present. Bowels moved freely.

July 8. Temperature 100°, otherwise no important change.

July 9. The note reads: "Temperature, pulse and respiration are normal. The patient is much brighter, headache has almost disappeared, the body is almost perfectly relaxed, patellar reflexes are normal, there is only a faint suggestion of Kernig's sign, the diplopia is less marked, but there is strabismus and paralysis of the left external rectus. The bowels have moved and the patient complains of hunger."

July 10. The urine contains a trace of albumen but no casts. Leucocytes 7.160.

July 11. Condition good, no discoverable rigidity, no headache, patient bright and hungry, tongue clean, temperature normal. Allowed to sit up.

July 12. All the symptoms except paralysis of external rectus and slight degree of diplopia have subsided; a suggestion of Kernig's sign remains. The diet is increased and the patient is allowed out of bed for a few minutes.

July 15. About ward.

July 18. Diplopia and Kernig's sign seem to be disappearing; still some paralysis of external rectus.

July 21. Feels well; can read without difficulty. No headache.

July 24. Improvement continues, paralysis of external rectus diminishing; all other symptoms have subsided.

July 26. "Discharged cured."

Discussion.—The first symptoms of meningitis appeared on July 2, and it is probable that lumbar punctures made at that time might have developed the nature of the disease. The symptoms increased in severity progressively until July 4 when they became marked. In view of the almost unavoidable uncertainty attaching to a determination of the onset of the disease it is safe to count July 3 to 4 as the first twenty-four hour period of its actual existence. Hence the serum was injected about 48 hours after the development of unmistakable symptoms. The symptoms abated rapidly after the second lumbar puncture and the injection of serum, and the patient experienced no relapses but improved progressively and made a complete recovery. Total amount of serum injected, 15 cubic centimeters.

Pennsylvania Hospital, Philadelphia. Service of Dr. J. C. Wilson.

CASE II. R. B. White male, aged 17 years. Clerk.

Present Illness.—On June 27, 1907, the patient was brought to the hospital in ambulance. He complained of malaise, severe headache, vomiting and constipation. The illness began on June 25 with malaise and headache. The same night the symptoms became suddenly much worse: headache, severe aches and pains of body, nausea and vomiting, chilly and feverish sensations, and alternating delirious and lucid periods until morning. The symptoms continued the next day and rigidity of neck was complained of. On admission temperature 100°, pulse 100, respirations 28.

Physical Examination.—Well-developed, robust-looking boy. Lies quietly in bed, although he tends to be restless and irritable; is rational but stuporous. Skin dry and hot; no rash; no herpes; face flushed; pupils slightly dilated and equal; react; conjunctivæ clear; tongue heavily coated and dry; head slightly retracted and rigid and painful. Kernig's sign marked; suggestion of Babinski reflex; patellar reflexes practically absent; some cutaneous hyperæsthesia; leucocytes 16,500 (nothing abnormal was discovered in heart, lungs and abdomen).

June 28. Condition essentially unchanged. The head is held more rigidly possibly and much headache is complained of.

June 29. Herpes appeared on lips; lumbar puncture at 3 p. m. and moderately turbid fluid obtained. *10 c.c. of antimeningitis serum injected* slowly into canal. No microorganisms found in the spinal fluid. One hour after the injection the temperature dropped from 100.6° to normal, the pulse became softer and ranged

from 74 to 64; respirations unchanged. Rigidity unchanged. In the evening temperature rose to 99.6°; patient very restless; morphia administered hypodermically.

June 30. Temperature 100.4°, dropping later to 98.4°; patient somewhat more comfortable; pulse 72. Evening: patient seems better, less pain and rigidity of head; has been sleeping or dozing much of the time; takes nourishment; bowels freely opened. Leucocytes 15,800.

July 1, 4.30 p. m. Lumbar puncture and 80 c.c. fluid removed; *15 c.c. antimeningitis serum injected.*—7 p. m. Temperature has risen from 98.5° to 101.5°. Patient perspired freely. No microorganisms found on cover-slips but many leucocytes. One colony of Gram-negative diplococcus grew on calf-serum agar.

July 2. Kernig's sign present; pain in back and legs; no headache; rigidity of head diminished. Leucocytes 10,550.

July 3. Has been very comfortable; no morphia given since July 1; no headache; rigidity of neck much lessened so that the head can be brought pretty well forward. Kernig's sign much diminished; no bodily discomfort; temperature normal or slightly subnormal.

July 4. Quite comfortable; hungry; given ice cream and orange juice. Kernig's sign diminishing.

July 6. Slight evidence of Kernig's sign; comfortable; on soft diet.

July 9. Up on bed rest.

July 16. Stiffness and pain in neck entirely and Kernig's sign practically gone. Temperature subnormal. Discharged.

Discussion.—The disease in this case began, probably, on June 25, the symptoms growing severe within 24 hours of their first appearance. The diagnosis of epidemic meningitis is rendered probable but not certain by the bacteriological examination. Lumbar puncture was performed and anti-meningitis serum injected twice. The first injection was made on approximately the third day, and the second on the sixth day of the disease. The recovery of the patient was progressive and complete without relapses.

Pennsylvania Hospital, Philadelphia. Service of Dr. Newlin.

CASE III. A. G. White female, aged 15 years. Canvas weaver.

Present History.—Went to work on September 6, but came home at noon on account of pain in head and back of neck. September 7 and 8 vomited several times. Since 6th inst. has been delirious and feverish and crying out. Admitted to hospital September 8.

Physical Examination.—Well-developed young girl lying in bed with chin thrown up and neck held stiffly; eyes injected; pupils equal, react; tongue heavily coated, dry; herpes about mouth; crying out; no rash. Kernig's sign well marked; knee jerks present, no ankle clonus. Nothing abnormal discovered in heart, lungs and abdomen.

September 9. Lumbar puncture yielded 3 c.c. of very turbid fluid containing *diplococcus intracellularis.* Leucocytes 23,000.

September 10. Condition unimproved. Kernig's sign and rigidity of neck more marked. Patient delirious, but can be made to give rational answers to questions.

September 11. Leucocytes 12,200. 50 c.c. of turbid, white fluid containing meningitis cocci withdrawn by lumbar puncture and *15 c.c. antimeningitis serum injected* into the spinal canal.

September 12. The note reads: "Twelve hours after the puncture and injection of the serum the temperature came down by crisis from 102.5° to 97° F.; the patient was quiet and sleeping soundly. The pulse was good and the respirations slow and regular. This morning the rigidity of the neck is lessened, the headache is less, the temperature subnormal, the respirations are regular and slow, the pulse slower (84) and of good volume, and the patient is improved in every way. The pupils are moderately dilated and the movements of the eyes good. There is a marked herpetic eruption about the mouth and three ulcers; one between fingers on the left hand and one on the left ear, and another beginning beneath the left eye. Leucocytes 8,750. "While counting the leucocytes six diplococci were found in a broken polymorphonuclear cell" (note by Dr. Longcope).

September 13. The temperature rose again yesterday, but came down during the night. This morning obtained 30 c.c. fluid by lumbar puncture and injected *15 c.c. antimeningitis serum.*

September 14. Much better to-day. Rigidity of neck and Kernig's sign almost gone; temperature subnormal; is rational.

September 15. Improving, but paralysis of the left external rectus is noted.

September 16. The paralysis of external rectus more noticeable and slight rigidity of neck and Kernig's sign still persist. 35 c.c. of clear, colorless fluid containing a few whitish flakes but showing no diplococci were obtained on lumbar puncture. *15 c.c. of antimeningitis serum injected.* Leucocytes 8,800. In the evening the temperature rose to 101°, the rate of respiration increased somewhat, but the pulse continued good (108). The rigidity of the neck and Kernig's sign were both increased, but they were much less marked than on admission.

September 18. Condition much improved. Neck rigidity and Kernig's sign have disappeared. Patient is hungry.

September 20. Temperature normal for three days.

September 24. Improving daily; house diet.

September 26. Out of bed.

September 30, discharged cured.

Discussion.—The onset of the disease in this case was abrupt, and the patient was admitted to the hospital about 48 hours after the appearance of symptoms. The diagnosis was established by the clinical symptoms and by lumbar puncture and a bacteriological examination on the third day of the disease, and a second puncture and an injection of the serum were made on the fourth day of the disease. It is worth noting that the circulating leucocytes fell to

one half the number between the first two punctures. A critical fall in the temperature took place after the second puncture and the first injection of the serum, and the general condition of the patient changed for the better while the rigidity of the neck muscles diminished. As the temperature rose slightly 72 hours after the first injection of serum a second puncture and serum injection were made. No further rise in temperature is recorded following the second injection of the serum. But as Kernig's sign and stiffness of the neck still persisted on September 16 and paralysis of the left external rectus appeared on the 15th, a third puncture and injection of serum were carried out. Since the leucocyte count was 8,800 on the day of the last injection complete previous cessation of active inflammation may be assumed, and the assumption is rendered certain by the limpid character of the spinal fluid withdrawn, from which all diplococci had disappeared. Forty-eight hours after the third puncture and serum injection all symptoms had subsided. The patient made a rapid and complete recovery.

Pennsylvania Hospital, Philadelphia. Service of Dr. Alfred Stengel.

CASE IV. C. B. White male, aged 11 years. Italian.

Present Illness.—The patient was admitted to the hospital on October 1 and had been sick since September 21. The onset was accompanied by vomiting, fever and chills. The complaint is of headache and pain in the back of the neck; the patient is delirious.

Physical Examination.—Half-grown, fairly well-nourished boy. He is very restless, requiring to be strapped in bed, and he cries out in delirium. Eyeballs are prominent; herpetic eruption about nose and mouth; tongue rough and covered with a brownish coat. The head is retracted and efforts to move it forward cause him to cry out. Suggestive Kernig sign; knee jerks unsatisfactory. Temperature on admission 101.2° F.

October 1. Leucocytes 10,500. 3 p. m. Lumbar puncture unsuccessful.—12.30 a. m. Second futile attempt to secure fluid by lumbar puncture. Patient very restless. Chloral and sodium bromide administered but ineffectual. Fairly quiet for 5 or 6 hours after ethyl chloride.

October 2, 4 p. m. Lumbar puncture yielded 6 c.c. thick, viscid, yellow pus. The last amount withdrawn thinner than the first. *10 c.c. of antimeningitis serum injected.* Pus contains many meningitis cocci. Kernig's sign positive. Patient very restless; muscular twitchings.

October 3. Leucocytes twenty hours after puncture 14,750. No relief from puncture and serum. Three hours after puncture temperature rose from 101° to 104° F. It remained at 104°, except for a temporary drop to 102° through the night and the next morning. Marked nystagmus this morning. Pulse weak and rapid (130–160), respiratory rate has been steadily increasing; muscular

twitchings are very marked and the arms are jerked spasmodically. Stimulation increased, but pulse and respiration grow steadily more rapid and feeble.—4 p. m. Lumbar puncture yielded 12 c.c. turbid, purulent fluid. *15 c.c. of antimeningitis serum injected.* Pulse and respiration gradually increasing. Temperature at 8.15, 106°. Unconscious and less noisy in delirium. Died at 1.45 a. m. (October 4). The autopsy (Dr. W. T. Longcope) showed acute cerebro-spinal leptomeningitis, acute broncho-pneumonia; chronic interstitial hepatitis; chronic interstitial splenitis; chronic fibrous pleurisy; congestion of intestine and hyperplasia of lymphoid follicles; and cloudy swelling of the kidneys.

Discussion.—The patient was admitted to the hospital on the tenth day of the disease. The puncture of the spinal canal indicated, and the autopsy proved, the cerebro-spinal meninges to be covered with a thick layer of pus cells and fibrin. No favorable influence was exercised on the course of the disease by the puncture and the serum injection carried out first on the eleventh and next on the twelfth day of the disease when the patient was already in an extreme condition.

Pennsylvania Hospital, Philadelphia. Service of Dr. Henry.

CASE V. P. C. Male, aged 18 years. Italian. Shoemaker.

Present Illness.—Patient came to the hospital on August 7 complaining of malaise, severe headache, chilliness and fever, nausea and constipation. The headache and malaise began on August 4 and increased so that he was compelled to stay in bed. On admission to the hospital the temperature was 101°, pulse 72, respirations 24.

Physical Examination.—Patient is moderately well-developed. He lies quietly in bed but is stuporous. Skin dry and hot; no rashes. Somewhat flushed and apathetic facies. Pupils equal and react sluggishly. Conjunctivæ injected. Tongue heavily coated and moist; breath fetid. The patient resents disturbance and becomes irritable; complains of pain in the head. (Nothing abnormal discovered in heart, lungs and abdomen. A blood examination showed Widal test negative and leucocytes 18,700. Slight degree of Kernig's sign present. Urine: no albumen, sugar or casts.

August 9. Temperature has almost reached normal; headache severe; there is marked rigidity of neck with slight retraction of the head. Some degree of rigidity of the back. The reflexes are exaggerated. Lumbar puncture was made at 1 p. m. but no fluid was obtained.

August 10. Temperature has risen. The condition of patient apparently worse; the stupor has increased and at times there is delirium. Rigidity of neck and Kernig's sign have increased; slight degree of nystagmus and strabismus. Lumbar puncture at 1 p. m. yielded 3 c.c. of spinal fluid tinged with blood. The centrifugated specimen showed polymorphonuclear leucocytes and red corpuscles, but no bacteria. Cultures were negative.

August 12. Condition essentially unchanged. The temperature fluctuated somewhat. Complains less of headache. Leucocytes 19,750. Lumbar puncture

at 1 p. m. yielded 4 c.c. of fluid of which 3 c.c. collected in one tube was slightly blood-tinged and 1 c.c. in another presented a silvery sheen. Cover-glass preparations showed many polynuclear cells, a few mononuclear cells and many red corpuscles, but neither tubercle bacilli nor diplococci. Cultures were sterile.

August 15. Patient slightly improved; brighter; temperature lower; still has considerable headache; persistence of neck rigidity and Kernig's sign; patellar reflexes exaggerated. Pulse good.

August 16. Condition about the same except that the temperature has become normal: Lumbar puncture at 1 p. m. yielded 7 c.c. of turbid spinal fluid. No tubercle bacilli found, but many pus cells and extracellular and smaller number of intracellular diplococci resembling the intracellularis, were seen. At 2.45 p. m. *15 c.c. of antimeningitis serum injected into the spinal canal.* "Patient stood the ordeal well, one hour later felt more comfortable and was dozing."

August 17. Temperature remains normal. Patient feels much better; he is brighter; headache less severe; neck less rigid, relaxing; Kernig's sign diminished.

August 19. Patient Convalescing. Headache slight; rigidity less; appetite improved.

August 22. Condition good; no headache; no rigidity; reflexes normal. Sitting up.

August 30. Discharged cured.

Discussion.—The diagnosis in this case must be accepted chiefly, if not entirely, upon the basis of the symptoms. The bacteriological examination of the fluid yielded by lumbar punctures does not supply convincing proof of the existence of epidemic meningitis. The degree of general leucocytosis is in agreement with what is commonly found in epidemic meningitis, but the evidence supplied by the leucocyte count is circumstantial. The examination of the sediment obtained from the spinal fluid showed an excess of polymorphonuclear cells, but it was not until the 16th instant, or on the tenth to twelfth day of the disease, that turbid spinal fluid, containing many leucocytes and stainable diplococci, was secured. It does not appear in the report that the cocci were certainly identified as *Diplococcus intracellularis* by culture tests. Hence the question remains open whether in this case the general cerebrospinal fluid remained free of the diplococcus although an exudative inflammation of some extent existed in the membranes. The case appears to have been progressing towards recovery before the injection of the serum on the tenth to twelfth day of illness, and there is lacking all certain evidence that the progress in this direction was accelerated appreciably by the serum. Total amount of serum injected, 15 cubic centimeters.

RESULT OF THE USE OF ANTISERUM BY DR. CUSHING, OF BALTIMORE.

Dr. Cushing wrote one of us (Flexner) as follows:

"I remember your telling me that you thought there was very little chance of benefit to be expected from the serum in other than the acute stages of epidemic meningitis. It may, therefore, interest you to know of this single experience. A woman, aged 36 years, had a severe attack of cerebro-spinal fever with sudden onset, May 16, 1907. She was very ill and during the next few weeks Futcher and a number of others saw her in consultation. The condition dragged on until July, when I was asked to see her in the hope that there might be some prospect of operative relief, since she was still suffering from irregular periods of fever (103°–104°) during which there were marked stupor, severe headache, and cervical retraction. She had been ill in all about eight weeks. Though under the impression that there was some mechanical obstruction with hydrops ventriculorum, a lumbar puncture during one of her stuporous periods evacuated a large amount of not particularly turbid fluid which was under high tension. To my astonishment the fluid was swarming with *Diplococcus intracellularis,* both inside and outside of cells. Forty-eight hours later, on the return of the stupor, another puncture was made and *15 c.c. of antimeningitis serum* were introduced into the spinal canal; the diplococci were still numerous. The temperature dropped to normal soon after. After a second forty-eight hours *this was repeated*; diplococci were few. Again in forty-eight hours there was a repetition of the puncture and *serum injection*; practically no diplococci could be found in the fluid, though there may have been a few organisms present. From this time on there were no further symptoms—no headache and no fever. She rapidly convalesced and has recovered her usual health."

Discussion.—The instructive points of this case relate to the persistence of the diplococcus in large numbers in the cerebro-spinal membranes for a number of weeks after the acute stage of the meningitis had passed off, and the action of the lumbar punctures and serum injections in interrupting and finally and quickly abating the free development of the diplococcus. Apparently the number of diplococci present at the second puncture, before any serum was injected, was not remarkably smaller than at the first puncture, while the next two notes describing the punctures and injections of serum dwell particularly on the diminution in numbers of the diplococcus. The subsidence of symptoms and return to perfect health were undoubtedly attendant upon and probably the outcome of the disappearance of the diplococcus from the cerebro-spinal membranes, and this disappearance was greatly aided by, and possibly accomplished through, the withdrawal of infected spinal fluid and the injection of the antiserum.

THE EPIDEMIC OF MENINGITIS IN GREAT BRITAIN.

A severe epidemic of cerebro-spinal meningitis, caused by *Diplococcus intracellularis,* prevailed in several cities in England, Scotland and Ireland during the winter of 1906–7, and is still prevailing in those countries. We have been fortunate in securing the co-operation in testing the antiserum of Dr. Claude Ker, of the City Hospital of Edinburgh, and of Dr. A. Gardner Robb, of the Belfast City Fever Hospital and the Belfast Union Fever Hospital, of Belfast. We have received preliminary reports, based on a small number of cases of meningitis treated with the antiserum, from these gentlemen which are given in this place. They will doubtless publish their experiences in full later, after a larger number of cases, treated with the antiserum, have come under their observation.

Dr. Ker writes under the date of September 17, 1907:

"Our experience with the serum has so far been limited to four cases. The first three have done well and seem now, after four and three weeks' treatment in hospital, to be out of danger. Our general impression here is that, although averagely acute cases, they have done most exceptionally well. One, however, I fear will be permanently deaf.

"The fourth case was fulminant with profuse hæmorrhages in the skin. It was admitted 18 hours from the onset and died about 24 hours from the onset. It was therefore hopeless, but I thought it just and right to give it the chance, and it had one dose of *30 c.c. of the serum,* which could hardly have had time to be effective. The other cases had about 120 c.c. on an average each. The only selection I am making is to treat cases which are less than a week old.

"By the way, my bacteriologist tells me that it is exceedingly difficult to get the diplococci to stain as usual the day after the first intraspinal injection. This has not been noticed so much with other serums. The germs are there of course and can be seen, but they must be modified in some way by the treatment, as they certainly lose their staining power."

Dr. Robb writes under the date of October 23, 1907:

"Since my return to duty on 1st September the opportunities of testing the serum have not been many, but the results in the cases receiving it have been remarkably satisfactory. I have only had four cases admitted to hospital quite early in the attack since that time.[4]

"The first case was a man of 22 who was 48 hours ill when admitted. He was wildly delirious with normal temperature, cyanosis and plentiful petechiæ. I considered his case practically hopeless from my former experience. I gave him

[4] In addition, two chronic cases of meningitis were treated, as appears from the latter part of the letter.

30 c.c. of the serum after drawing off 90 c.c. of turbid fluid in which the meningococcus was present. 36 hours later he was quite conscious and had no headache; his temperature rose to 101° F. and remained about that level for some days; he had very abundant herpes, but his symptoms rapidly improved. I repeated the *30 c.c. injections at intervals of 3 days,* giving 90 c.c. in all. He made a complete recovery. The remarkably sudden clearance of the mental symptoms and the complete disappearance of the headache were very striking, and as this has taken place in other cases treated with the serum, I think the serum must have the credit.

"The second acute case was a girl of 12 years with very severe attack admitted on the second day. This case was very severe, but the prognosis without serum would have been uncertain. The same good results followed with good recovery.

"The third case, a woman of 31, had been ill 10 days with very severe attack; high temperature, delirium, and very marked rigidity. My prognosis would have been bad, but she rapidly improved after the serum and is in a fair way to recovery now. She had 90 c.c.

"The fourth acute case, a man of 20, with maniacal delirium, came in two days ago. I gave him *30 c.c. of serum* immediately on admission—then 46 hours ill. The cerebro-spinal fluid was quite purulent even then. He died of heart failure some 9 hours after receiving the serum.

"Even more striking were the results in two chronic cases. One boy not doing well, who had continuous fever for 35 days, received *30 c.c. of the serum.* From that time on he made steady improvement, had no further headache and has made a complete recovery. Another case which I considered quite hopeless got 30 c.c. on the 25th, and again on the 32d day. He made steady improvement from the first dose and is now up. One young child, who was also hopeless, showed no improvement and died, but in this case thick, stringy pus was obtained. In the chronic cases no improvement had followed simple drawing off of fluid.

"I quite appreciate how dangerous it is to draw conclusions from a few cases, especially during a lull in the epidemic, but allowing for all that I am greatly impressed with the results in the cases I have had. I believe there has been little if any change in the virulence of the type here. The cessation of the headache in the chronic cases receiving serum and in whom it had been most troublesome was very striking; and the absence of headache in the acute cases after serum I have not seen in any other cases."

Discussion.—There could be no advantage gained in discussing these cases, since they are reported so very briefly. Attention may, however, be called properly to the effects of the serum injections in the two cases of the chronic disease described by Dr. Robb. They recall the similar effects of the serum injections made in the chronic case of epidemic meningitis described by Dr. Cushing, of Baltimore.

ST. VINCENT'S HOSPITAL CASES, NEW YORK CITY.

The antiserum was not available for use in the treatment of meningitis during the prevalence of the severe epidemic in New York City. Since it has been available only occasional sporadic cases of the disease have continued to appear, and many of these have entered hospitals late in the course of the disease. Hence our opportunities for testing the antiserum under conditions of personal observation have been very few. We have secured from Dr. Strain, of St. Vincent's Hospital, the records of three cases of meningitis in adults caused by *Diplococcus intracellularis* of which abstracts follow.

CASE I, No history of onset obtained; total serum injection 30 c.c.; death.
CASE II, 1st injection of serum on 4th day; total serum injection 30 c.c.; recovered by lysis.
CASE III, No history of onset obtained; total serum injection, 60 c.c.; recovered by lysis.

St. Vincent's Hospital, New York. Service of Dr. Lewis.
CASE I. A. C., aged 22 years. Greek.

Present Illness.—No history could be obtained. Admitted to hospital, April 23, 1907.

Physical Examination.—The patient is in a semi-comatose condition. Pupils unequal; right pupil does not react to light; left is dilated; no strabismus. Herpes on upper lip. Marked retraction of the head and stiffness and tenderness of neck. Reflexes absent from extremities; Kernig's sign present. Babinski reflex absent.

April 23. Patient is somnolent, pulse slow and of medium tension; general condition poor. Temperature ranged from 102°–100°.

April 24. Patient noisy, crying out. No remarkable change. Leucocytes 18,000.

April 25. General condition about the same. 15 c.c. of turbid spinal fluid obtained by lumbar puncture. Microscopical examination showed pus cells and *Diplococcus intracellularis.*

April 26. Condition worse; the pulse is rising gradually and the first heart sound is becoming weaker; capillary circulation very poor; cyanosis; cold extremities. Temperature 100°–103°; pulse 99–112; respirations 28–34.

April 27. Condition about as yesterday. 20 c.c. of turbid spinal fluid removed by lumbar puncture and *30 c.c. of antimeningitis serum injected.* Following this the temperature rose 1° and then gradually declined during the night to 99°, the patient's condition gradually becoming worse.

April 28. Condition very bad; pulse hardly perceptible; respiration accelerated; physical signs unchanged.

April 29. Lumbar puncture unsuccessful. Condition bad.

May 1. Death at 1 a. m.

Discussion.—The duration of the disease in this case is not known. The diagnosis of epidemic meningitis, which was suggested by the symptoms, was established by lumbar puncture on April 25, two days after admission to hospital. The second puncture and the first injection of the serum were made on April 27, four days after admission to the hospital and at a time when the patient's condition was already very poor and the circulation had begun to fail markedly. No improvement followed the puncture and injection, but the patient's condition gradually grew worse until his death on May 1, eight days after admission to the hospital, four days after the serum injection, and an unknown period after the onset of the disease. Total amount of serum injected, 30 cubic centimeters.

St. Vincent's Hospital, New York. Service of Dr. Lewis.

CASE II. C. L. Aged 22 years. Greek. Laborer.

Present Illness.—On May 7 (one day before admission to hospital) the patient was taken suddenly ill with sharp chill and intense headache. The neck became stiff, and slight movement greatly increased pain in head and neck. There were fever and photophobia.

Physical Examination.—Flushed face; anxious expression; injected conjunctivæ; photophobia; beginning herpes on nose and lips; tongue coated; breath fœtid. Neck stiff and head retracted. Kernig's sign marked; reflexes absent; skin hyperæsthesia.

May 8. Temperature 102°, pulse 90, respirations 24 on admission. Patient quiet, but complains of headache. Under ice cap slept much of the day.

May 9. Patient somnolent; cries out at times. Temperature ranged from 103.4° to 105°; pulse 80–94.

May 10. At 9 a. m. and 1 p. m. temperature 104°.—4 p. m., lumbar puncture yielded 35 c.c. of turbid fluid containing pus cells and large numbers of diplococci. Headache better; slept much of night.—5 a. m. Temperature 100°.

May 11, 4 p. m. Lumbar puncture; 30 c.c. spinal fluid withdrawn and *30 c.c. of antiserum injected.* During the night patient was very noisy and required sedatives (morphia and hyoscin) to give sleep.

May 12, 9 a. m. Temperature 104°; remained high all day. No marked change in the condition.

May 13, 5 a. m. Temperature 100°. Pulse slow and full; mental condition apathetic. Temperature did not rise above 101°. Involuntary bladder and bowel movements.

May 14. Condition improved; mental condition brighter. Patient follows with the eyes persons and objects about the room.

May 15. No marked change. Temperature ranged from 101.8° to 103°.

May 16. Condition better. Awake greater part of day; headache less; does not complain of pain in the neck.

May 17. Improvement continues; patient comfortable; mentally brighter; less rigidity of neck; appetite improved.

May 19. Temperature normal; neck rigidity and headache almost gone; patient bright and cheerful.

The improvement in the patient's condition continued and he was discharged cured on June 8.

The leucocyte count on three occasions gave 11,000, 15,000 and 14,000.

Discussion.—The onset of the symptoms in this case was sudden and by the end of the first twenty-four hour period the disease had reached full development. On the third day, counted from the appearance of the first symptoms, lumbar puncture was made and the diagnosis established by bacteriological examination. The temperature fell, the headache diminished, and the patient rested better than before. On the fourth day a second puncture was made and the antiserum was injected. No immediate favorable effect followed, but 60 hours later the condition had improved and there was a steady subsequent inprovement up to complete recovery. Convalescence may be said to have begun not later than the tenth day of the disease. Total amount of serum injected, 30 cubic centimeters.

St. Vincent's Hospital, New York. Service of Dr. Lewis.

CASE III. G. P. Aged 18 years. Greek. Clerk.

Present Illness.—No definite history obtainable. About a week before admission to hospital on May 18, 1907, he complained of headache and vomiting.

Physical Examination.—Face flushed and expresses pain; photophobia; pupils equal and react to light; tongue coated and dry; neck rigid and head retracted; a few petechial spots on thorax, abdomen and legs; reflexes exaggerated; no clonus or Babinski; Kernig's sign marked. May 18 the temperature rose from 98.5° to 103°; pulse 85; respirations 28.

May 19–28. The condition has not greatly altered. The mental condition dull, there is much headache and at times delirium requiring restraint. The temperature did not rise above 102.5° and ranged about 101°. Leucocytes 24,000.

May 27. Leucocytes 21,000.

May 29. Patient quiet and somnolent. By lumbar puncture about 50 c.c. of turbid fluid were obtained and *30 c.c. of antiserum injected.* Following the injection the patient was quiet and the temperature fell to 99°. Microscopical examination of spinal fluid showed pus cells and intra- and extracellular diplococci, which were cultivated.

May 30 and 31. Mental condition somewhat improved; no delirium.

June 1. External strabismus appeared, otherwise no change.

June 2. Mental condition clearer; patient answers questions. Temperature fluctuating.

June 4. Patient complains of pain in neck and is at times somewhat delirious. About 50 c.c. of spinal fluid removed by lumbar puncture and *30 c.c. antimeningitis serum injected.* The temperature fell from 103° to normal in twenty-four hours. Delirium subsided and patient looked brighter. He had involuntary defecation and micturition.

June 6. Leucocytes 20,000.

June 7. Patient noisy at night, requiring sedatives.

June 10. Pulse weak but responds to digitalin.

June 11. Neck rigidity lessened; patient fairly comfortable; photophobia and strabismus gone; diet increased; temperature normal.

June 15. Patient gradually getting stronger; mental condition good.

June 18. Improvement continues.

June 21. Patient noisy and delirious all day; temperature 99°. For the next two or three days he was delirious at times.

June 27. Patient rational all day and slept and ate well. Temperature 97°.

June 29. Patient sat up for the first time.

July 5. Walked a few steps; leucocytes 6,000.

July 20. Discharged cured.

Discussion.—It is impossible to determine accurately the duration of the disease before the patient entered the hospital. On the eleventh day after admission lumbar puncture was made and a serum injection was given. Following these the patient's mental condition improved somewhat, but three days later external strabismus appeared. On the seventeenth day of illness a second lumbar puncture and serum injection were made which were followed within twenty-four hours by a marked fall in temperature and an improvement in the patient's mental condition. The temperature remained about normal afterwards, although there reappeared off and on for a few days, during the convalescence, a state of temporary delirium, which did not interrupt the general course of improvements in the patient's condition. Recovery was complete. Whether the serum injections influenced favorably the progress of the disease in this case cannot be determined with certainty. Total amount of serum injection, 60 cubic centimeters.

MANNER OF ACTION AND OF EMPLOYING THE ANTISERUM.

The plan to administer the antiserum by direct inoculation into the spinal canal in human beings was based upon the observations made by one of us (Flexner) on the bactericidal effect of normal sera and sterile exudates upon *Diplococcus intracellularis in vitro,*

and upon the curative action of antidiplococcus sera in guinea-pigs and monkeys infected with the diplococcus, when brought into immediate relation with the focus of infection. In view of certain theoretical objections to the employment as curative agents of antisera developed for a microorganism whose toxic action is caused by endotoxin, Flexner dwelt on the encouraging circumstance that in epidemic meningitis the main pathological lesions can be brought directly under the influence of the antiserum by injecting the latter into the spinal canal; and he pointed out that while it is undoubtedly important to secure neutralization of the endotoxin yielded by the diplococcus on disintegration, the effect of restraint of growth and multiplication of the diplococcus may, at some period of the disease, be of even greater significance.[5] There is experimental evidence for the view that the antiserum possesses a certain antitoxic value since it can neutralize the toxic substances contained in autolysates of the diplococcus. But its power to bring about rapid suppression of the diplococcus in infected guinea-pigs and monkeys is considerable. In monkeys which have been injected with mixtures of emulsions of the diplococcus and immune serum simultaneously, or first with the emulsion and next with the immune serum, the diplococcus is caused rapidly to diminish in numbers and to be more abundantly taken up by leucocytes.[6] Since the facts at hand do not warrant us in concluding that any considerable multiplication of the diplococcus takes place in the experimental infections, the power of protection of the antiserum would appear to be dependent upon the restraint which it exercises over all multiplication and the increased tempo of phagocytic inclusion of the diplococcus which it brings about. It is probable that phagocytic digestion not only prevents further multiplication of the diplococcus but also that it detoxicates the endotoxin by reducing it to simpler and non-toxic or less toxic compounds. Still, in a few instances, in which the antiserum was injected into the spinal canal of monkeys infected with the diplococcus, the microorganisms disappeared without marked phagosis and more slowly than in the cases in which outpouring of

[5] *Jour. of Exper. Med.*, 1907, ix, 138.
[6] *Idem*, p. 169 *et seq.*

leucocytes was considerable. The control of the pathological conditions in these instances appeared to depend less upon the phagocytes than upon the spinal fluid reinforced by the antiserum; and as the symptoms of intoxication were less than would have been present had the antiserum not been injected, a degree of antitoxic power must be ascribed to the antiserum.

If we turn to our knowledge of the manner in which the antiserum acts in controlling or modifying the infection in human beings we find ourselves possessed of very few facts. The observation recorded by Dr. Cushing indicates that the antiserum has the property of bringing about rapid diminution in the number of diplococci present in the cerebro-spinal fluid. Dr. Ker observed rapid rise of the opsonic index for the diplococcus following upon an antiserum injection and a modification and reduction of the staining power of the diplococcus in film preparations prepared from the meningeal exudate obtained by lumbar puncture. Others have noted this reduction in number and change in the staining properties of the diplococcus after the serum injections.

We have had the opportunity to follow in two young children the immediate effects of the antiserum injections on the number, appearances and viability of the diplococcus in the cerebro-spinal fluid. One of the children was 18 months old and had been two weeks ill when first injected at the Babies Hospital in New York. The spinal fluid withdrawn before the serum injection was slightly turbid and showed a fair number of extracellular and a large number of intracellular, sharply-staining diplococci. Cultures were easily secured from this fluid on blood and sheep-serum agar media. A second puncture made twenty-four hours after an antiserum injection yielded a fluid of the same appearance as before, but no extracellular diplococci, or very few, were contained in it, and the number of intracellular diplococci was much reduced. All the diplococci were more or less changed; they were swollen or fragmented and stained diffusely. Cultures were now negative. A second antiserum injection was given and the next day the diplococci had undergone a still greater reduction in numbers and continued to stain feebly. No cultures could be obtained from this fluid or any subsequent fluid from this child, although several later punctures were made.

The second child was two years old and had been ill about five days when admitted to the Presbyterian Hospital in New York under Dr. Northrup. The first lumbar puncture yielded a sero-purulent fluid containing large numbers of diplococci outside and inside pus cells. Abundant cultures mere easily obtained. An injection of 15 cubic centimeters of antiserum was given and twenty-four hours later the lumbar puncture yielded a fluid in which a little blood obscured the color, but the pus cells and diplococci were diminished in numbers. The latter were now almost wholly inside cells and of irregular size and contour and weak staining power. A portion of the fluid was centrifugalized and the sediment used for preparing cultures which did not grow. Serum injection and lumbar puncture were repeated on two later occasions. The spinal fluid withdrawn was far less purulent than it had been, the diplococci became very few in number, and they did not again multiply on culture media otherwise favorably adapted for the growth of the diplococcus.

These observations, few in number as they are, go to show that the antiserum exerts a definite and injurious influence upon the diplococcus in the cerbro-spinal fluid through which its multiplication is restrained and it is rendered more subject, possibly, to phagocytic inclusion and digestion, at the same time that it is deprived of its capacity to grow outside the body on culture media.

We have given the following general instructions for the use of the serum:

The antiserum should be kept in a refrigerator until it is to be used, when it should be warmed to the body temperature before it is injected.

The antiserum is to be introduced directly into the spinal canal after the withdrawal of cerebro-spinal fluid by means of lumbar puncture.

The quantity of antiserum to be used at a single injection should not exceed, for the present, 30 cubic centimeters. It is desirable, although it would not appear to be essential, to withdraw from the spinal canal at least as much fluid as the amount of antiserum to be injected. The injection should be made slowly and carefully to avoid the production of symptoms due to increased pressure. This

precaution should be exercised especially where the quantity of cerebro-spinal fluid withdrawn is less than the amount of antiserum to be injected.

The injection of the antiserum should be repeated every twenty-four hours for three or four days or longer. Whether any advantage will be gained by more frequent or more numerous injections than here indiciated a wider experience must decide. As much as 120 cubic centimeters of the antiserum have been injected into the spinal canal in four days without causing unpleasant symptoms.

The evidence at hand indicates that the earlier in the course of the disease the injections are made the better the results. Hence should the film preparation prepared from the first fluid obtained by spinal puncture show Gram-negative diplococci, some of which are within leucocytes, an injection of the antiserum should be made immediately and without waiting for the result of culture tests. Should the diagnosis be left in doubt or the disease prove later to be of another nature than epidemic meningitis, no harm will have been done by the injection of the antiserum.

Although the best results have thus far been obtained where the antiserum has been injected early in the disease, yet the serum should be used in its later stages also until our knowledge governing the value of the serum becomes more precise. The indications at present are that it is useless to employ the serum in the very late stages of the disease in which chronic hydrocephalus is already developed.

Precise records of the manner of action of the antiserum upon the general symptoms of the disease and the local inflammation and the diplococcus should be kept. Information is greatly desired on the influence of the antiserum upon the number, appearances, growing properties, etc., of the diplococcus, upon the relation of the diplococcus to phagocytosis, and on the number and appearances of the leucocytes, before and after the antiserum injections. Counting the leucocytes in the circulating blood, before and after the injections, would help determine whether the antiserum tends to bring a greater number of leucocytes into the inflamed membranes, or whether it leaves the number unchanged or causes cessation of the emigration.

Until the antiserum is proven to be of value or of no value in the treatment of epidemic meningitis its manner of action should be carefully observed and recorded so that a definite decision may be reached as quickly as possible.

DOES ANAPHALAXIS OCCUR FROM REPEATED INJECTIONS OF THE SERUM?

That the human organism reacts more vigorously to second and subsequent injections of horse serum than to the first injection is shown by the reports of many instances in which these stronger effects were noted after administering diphtheria antitoxin. v. Pirquet and Shick[7] call this condition of greater reaction on the part of the animal organism "serum-disease." Wolf-Eisner[8] sees in this state of intensified effect or hypersensibility the fundamental pathological condition underlying the manner of reaction of the animal body to repeated injections of foreign proteids in general, including the bacterial endotoxines. Our precise knowledge of serum-hypersensibility—or anaphalaxis—is due to the impulse given the study of the subject by Theobald Smith and to the exact studies of Otto,[9] Rosenau and Anderson,[10] Besredka and Steinhardt,[11] Gay and Southard,[12] Lewis[13] and others. The particular fact that concerns us at this moment is whether a possible danger to the patient is to be feared from intraspinal injection at considerable intervals of a foreign serum. We know that the intensified effects in man of repeated serum injections under the skin causes discomfort but does not menace life. Besredka and Steinhardt have, however, shown that it is precisely the direct inoculation of the central nervous system with the alien serum in a hypersensitive guinea-pig that is to be feared. It is, therefore, of the first importance to us to ascertain whether a similar danger exists in relation to the intradural injection of the antimeningitis serum.

[7] Die Serumkrankheit, Vienna, 1905.
[8] *Berl. Klin. Woch.*, 1907, xliv, 38.
[9] *Leuthold-Gedenkschrift*, 1906, i, pt. 1, 153.
[10] *U. S. Marine Hosp. Service Hygiene Lab. Bull.*, 1906, No. 29.
[11] *Annales de l'Institut Pasteur*, 1907, xxi, 117, 384.
[12] *Jour. of Medical Research*, 1907, xi, 143.
[13] *Jour. of Exper. Med.*, this number.

There is no danger, apparently, to be apprehended from a single injection of even a considerable volume of the serum into the spinal canal. Daily intradural injection of the antiserum seem also to be well borne, at least, for several days. The question arises whether it is safe to give the injections at intervals of many days, since an interval is necessary in order that the reaction of hypersensibility shall be developed. Dr. Ladd's Case V, a child of three years, was injected on the following dates: April 1, 6, 22; no ill effects followed and recovery was complete. His Case XV, a child of eight years, was injected as follows: June 16 intradurally, June 18 idem, June 21 subcutaneously, June 25 idem, June 27 idem, July 10 intradurally; no ill effects followed and the child recovered. Still other instances of repeated injection, with intervening long interval between certain injections, will be found among the recorded cases. The danger does not, therefore, seem to be great.

We wish now to refer to an infant who was injected at the Babies Hospital, New York City, several times with the antiserum. The fourth injection was made 42 days after the first and 16 days after the third injection and was followed by convulsions, prolonged rigidity and elevation of temperature.

Babies Hospital, New York. Service of Dr. L. Emmett Holt.

E. F. Female child, 11 months old. About the middle of August developed fever, hyperæsthesia, rigidity and projectile vomiting. Admitted to Babies Hospital September 6, 1907. Lumbar punctures on September 7 and 11: turbid fluid withdrawn. September 12, 45 c.c. fluid withdrawn and *5 c.c. antiserum injected.* September 17, 25 c.c. fluid withdrawn, no injection. September 28, 7 c.c. fluid withdrawn and *7 c.c. antiserum injected.* October 8, 3 c.c. fluid withdrawn and *5 c.c. antiserum injected.* No symptoms developed following these injections. October 24, 60 c.c. fluid were withdrawn and *20 c.c. antiserum injected.* Previous to the last injection the child was relaxed and opisthotonos was absent. She was restless and irritable, vomited occasionally, cried if disturbed, and was apparently deaf. The reflexes were increased and there was marked emaciation. The antiserum was administered intradurally at 11 a. m. At 3 p. m. a severe convulsion occurred and marked hyperextension and opisthotonos developed. These were still present on October 29. The temperature before the injection was about 98 to 99°; for four days after the injection it rose to 102° and once reached 104°.

The conditions which developed in this child following the last injection of serum, after an interval of 16 days since the preceding injection, cannot be explained readily. We prefer to leave the

question open whether the phenomena belong to the anaphalactic state or are of another nature. But so far as the case bears on the general question of the intradural injection of the serum it has theoretical rather than practical significance. The antiserum will, as a rule, discharge its beneficial effects in the first days of its employment and for this no danger is known to exist. Rarely, after a resting period in its use, a relapse of the acute infection may call for another injection. The records of the use of the antiserum in relapses do not show that any ill effects followed the injections. We think that the reinjection of the serum in supposed relapses should be based upon demonstrated reappearance of or increase in the diplococcus, since mere sudden rise in the temperature, in the course of meningitis, may obviously be due to other causes than a reinvasion of *Diplococcus intracellularis.*

MANNER OF PREPARING THE ANTISERUM.

The antimeningitis serum employed in the treatment of the cases of epidemic meningitis described in this paper has been made in the horse. The general method of preparation has been as follows:

The first inoculation consisted of cultures of the diplococcus, heated to 60° C. for 30 minutes, injected under the skin. Many different strains of the diplococcus were combined to prepare this vaccine. The first dose was the equivalent of ¼ surface growth on sheep-serum agar in a test tube. The dose was doubled at each subsequent inoculation, until an amount equal to four test tube growths could be given at 5 to 7 day intervals.

Intravenous inoculation was now substituted for the subcutaneous. Beginning with one oese of living diplococci the dose was progressively increased to 2, 3, 5, etc., oese, then ½, ¾, 1, etc., agar slant cultures, and finally to 1½ bottles (12 oz. Blake) of surface growth. The larger quantities of the culture injected into the vein caused such severe reactions and alarming symptoms that they were discontinued.

Subcutaneous and intravenous injection of an autolysate[14] was now used. The doses, at first 1 cubic centimeter, were later increased to 3 cubic centimeters. The injections were made about one week

[14] *Jour. of Exper. Med.*, 1907, ix, 105.

apart. The intravenous injection of the autolysate was discontinued because of the serious symptoms (increased respiration, weakness, etc.) which resulted from them.

At the present time the subcutaneous tissues are being used exclusively for the inoculations, which are made alternately of living diplococci and autolysate at 7-day intervals. Many different strains of the diplococcus are employed in preparing the living cultures and the autolysate for inoculation. The dose of living diplococcus has been increased to one and one half bottles, and of autolysate to the equivalent of one and one half bottles of the cultures.

The febrile reaction to the subcutaneous inoculations is moderate: The temperature rises to 39° to 39.6°. The animal eats less during the febrile period. The local reaction is much greater. Within a few hours a swelling appears at the site of inoculation and extends widely—from the shoulder to the knees at times. The swelling tends to disappear in a few days or, in those instances in which the larger doses of living diplococcus or autolysate are used, sterile abscesses develop which eventually discharge through the skin.

The antiserum has been titrated by the complement-deviation method devised by Kolle and Wassermann,[15] and tested against the autolysate in guinea-pigs. Neither method appears to be quantitatively accurate. The antiserum used in the treatment of the cases of epidemic meningitis described in this paper came from a horse in process of immunization one year or longer before being used for supplying the serum.

Coincidently with our efforts to produce an antidiplococcus serum for use in human beings suffering from epidemic meningitis, Kolle and Wassermann[16] and Jochmann[17] attempted in Germany to prepare such a serum. Only brief reports of the employment of these sera in the treatment of epidemic meningitis have thus far appeared in print. Wassermann[18] has recently reported the results of the treatment of a series of cases of the disease by subcutaneous injections chiefly of his serum, with results which, on the whole, appear

[15] *Deut. med. Woch.*, 1906, xxxii, 16.
[16] *Ibid.*
[17] *Ibid.*, p. 20.
[18] *Ibid.*, 1907, xxxiii, 1585.

to be favorable to the value of the treatment. Schöne[19] treated a still smaller series of cases with Jochmann's serum, partly by the subcutaneous and partly by the intradural method of injection, with results said to have been beneficial, especially where the injections were made into the spinal canal.

GENERAL DISCUSSION.

In view of the small number of cases of epidemic meningitis upon which this paper is based, it would seem the wisest policy, possibly, to defer all general discussion of the results until such time as a larger series of cases treated with the antiserum having been collected, a searching analysis can be made.

On the other hand it seems desirable to recapitulate, at this time, the salient points brought out by the different reports which have been collected in this paper, it being understood that they do not represent conclusions but merely statements of observed facts or obvious deductions from them.

Thus, for example, the records of cases used in the preparation of this paper show that of 47 cases of epidemic meningitis treated with the antiserum, 34 recovered and 13 died. Expressed in percentages, the recoveries equal 72.3 and the deaths 27.6 per cent. The records also show that of the 13 fatal cases four were either fulminant in type, in which death took place within 24 to 36 hours of the onset, or the patient's condition was so extreme that death occurred in a few hours of the injections of the antiserum. If, therefore, these four cases are subtracted there results a total of 43 cases of meningitis treated with the antiserum of which 34 recovered and 9 died, or 79.9 per cent. recoveries and 20.1 per cent. deaths.

This tabulation takes the cases without respect to their duration at the time the treatment was begun. This is manifestly a severe test, but so long as the whole number of cases is so small no other mode of analysis is likely to be as useful. Should it be desired, however, to know the ratio of recoveries to deaths in cases injected with the antiserum in the first three days of illness, the calculation could be based on 18 cases of infection, exclusive of the ful-

[19] *Die Therapie der Gegenwart,* 1907, xlviii, 52.

minant ones, of which 16 or 88.9 per cent. recovered and 2 or 11.1 per cent. died.

In order that these figures should have any value whatever we are obliged to know with what degree of severity the epidemic prevailed at the time and place at which the antiserum was used. We possess, fortunately, good data concerning this point. Eighteen cases of epidemic meningitis occurred at Castalia within a few weeks. Of these twelve patients died and six recovered. Of those patients who recovered three were injected with the antiserum, and no patient having received the serum died.

The report from Akron covers twenty cases of epidemic meningitis of which nine were not treated with the antiserum: Eight died and one recovered. The remaining eleven cases were treated with the antiserum: three died and eight recovered. The three fatal cases included two of the fulminating variety.

Dr. Robb[20] has given statistics covering 230 cases of epidemic meningitis which arose in Belfast. Of these 162 died making a mortality of 70.43 per cent. As regards the severity of the disease at the time at which the antiserum prepared by us was employed he states in his letter (p. 203): "I believe there has been little if any change in the type here."

The majority of the cases which were treated with the antiserum were in children over five years of age and in adults. Since the mortality is highest among young children a table has been prepared, from Dr. Ladd's report, giving the results of the use of the serum in children under five years of age (p. 185). Of the eight children belonging to this group seven were under three years and one was about five years old. Five of the children recovered and three died. Two of the three fatal cases were injected on the fourth day and one on the twentieth day.

The records of the patients who recovered have been full enough in twenty-five instances to enable us to make out the manner of termination of the disease—whether by lysis or by crisis. We have found that thirteen times the disease terminated by lysis and twelve times by crisis. The accompanying table (Table I) illustrates this point and enables the individual case-histories, upon

[20] *British Medical Journal*, 1907, No. 2443, 1129.

TABLE I.

Dr. Ladd's Cases.

Case No.	Day of Disease. First Injection.	Total c.c. Anti-serum Injected.	Recovered by Lysis.	Recovered by Crisis.	Died.
I.	3	25	+		
II.	12	5		+	
III.	4	30	+		
IV.	5	15			+
V.	2	35		+	
VI.	1	15		+	
VII.	3	20		+	
VIII.	3	45	+		
IX.	2	35			+
X.	1	15		+	
XI.	20	25			+
XII.	4	46			+
XIII.	1	45		+	
XIV.	14	28	+		
XV.	3	53	+		
XVI.	4	41			+
Akron Cases.					
I.	5	82.5	+		
II.	1	10			+ F
III.	12	20	+		
IV.	2	43.5	+		
V.	7	25		+	
VI	2	22.5			+
VII.	1	15		+	
VIII.	1	35			+ F
IX.	2	105	+		
X.	1	25		+	
XI.	2	22.5		+	
Pennsylvania Hospital Cases.					
I.	4	15		+	
II.	3	25	+		
III.	4	45		+	+
IV.	11	25			
V.	10	15	+		
St. Vincent's Hospital Cases.					
I.	?	30			+
II.	4	30	+		
III.	?	60	+		
Edinburgh Cases. Recovered (no details).					
I.	—7	120	+		
II.	—7	120	+		
III.	—7	120	+		
IV.	1	30			+ F

Belfast Cases.					
I.	2	90	+		
II.	2	90	+		
III.	10	90	+		
IV.	2	30			+ F
V.	35	30	+		
VI.	25	60	+		
VII.	?	?			+
Dr. Cushings' Case.					
I.	56	45	+		

— = less than. F = Fulminating.

which it is based, to be scrutinized. In a number of cases the abrupt termination of the disease, after acute and violent onset, within forty-eight hours of the injection of the antiserum, was striking and impressive. In some cases the serum was injected as early as twelve hours, and in one case as early as two hours, after the onset of severe symptoms, with prompt arrest of the disease.

Once or twice after abrupt arrest of active symptoms lasting several days, relapses occurred which were as promptly controlled by another injection of serum as was the original infection. It would appear that during the height of an epidemic of meningitis spontaneously abortive cases are of infrequent occurrence.

To discuss, on the basis of so small a series of cases as is here presented, whether the antiserum can be said certainly to influence favorably the temperature, mental condition, and such special symptoms as headache, muscular rigidity, paralyses, etc., seems hardly worth while. Moreover an analysis of the reports having these points in view can be made far better by those who have been in daily contact with the ill from epidemic meningitis and have learned, at first hand, to know its protean aspects and variable course. But, on the other hand, it is patent that the successful cases reported here have, almost without exception, made complete and rapid recoveries. There have been few or no long and tedious periods of convalescence, and in one instance only has a permanent defect—in this instance some imparement of hearing—remained.

Our choice of mode of introducing the antiserum into the body, namely into the spinal canal, should be justified. We were led to this manner of employment by two considerations: First, the

theoretical advantage of bringing the antiserum into direct contact with the focus of infection and inflammation, to support which we possess data based on animal experimentation; and second, the knowledge that elimination of colloids, and of crystalloids even, from the blood stream into the cerebro-spinal fluid is a slow and imperfect process in health and probably in inflamed states of the membranes also. Since there appeared to be no danger from this method of introduction of the serum, provided care was exercised, it was chosen; and the cases recorded in this paper bear testimony to its safety. In a few instances the subcutaneous has been superadded to the intradural injection, but whether any advantage is to be derived from such double injections, greater experience will have to determine. Could the subcutaneous be substituted for the intradural method there would obviously be a gain in convenience and probably in safety of general employment of the serum. We think it improbable that the results would be as good by the subcutaneous as they appear to be by the intradural method.

We possess evidence that the direct effect of the antiserum upon the diplococci present in the exudate in the cerebro-spinal membrane is to cause their rapid degeneration and an arrest of their free multiplication. This must be of some advantage to the patient. We have noted remarkable reduction in number and striking evidences of degeneration and loss of power of growth of the diplococcus, twenty-four hours after an injection of the antiserum. An exudate previously sero-purulent may be converted into a merely turbid exudate by an intradural injection of the serum.

The few cases in which the exudate has been really purulent or fibrino-purulent and present in small quantity, as judged by the few drops which could be secured on lumbar puncture, seemed not to be benefited by the serum injections. Whether such cases are very unpromising will have to be determined by wider experience with the antiserum.

Although we have discussed, briefly, the question of anaphalaxis, the cases in which several injections of serum were made, sometimes with considerable intervals between the injections, tend to show that the danger is not a very real or impending one. It can, we think, be neglected in practice for the present.

It is clear that once we accept the fact of the abortive and critically terminating cases as being caused by the antiserum, the early injection of it is to be sedulously sought. The figures, small as they are, also point to better results from the injections made in the first three days of the illness as compared with those made at a later period. And yet favorable effects have been recorded in cases treated in the fourth, fifth and eighth week of the disease. Basing a tentative deduction on the few facts of our present knowledge, we may suppose that as long as living and multiplying diplococci remain in the membranes and their fluid, the serum may be employed with hope or expectation of useful results. The serum is clearly of no avail in the treatment of symptoms resulting from chronic obstructive lesions of the membranes.

The doses of the serum employed thus far rest on an empirical basis. Whether the larger doses which have been used latterly are more efficient than the smaller ones used at first, can be determined only by a wider experience than we have yet had. In test-tube experiments degree of concentration of serum plays an important part in determining injury and disintegration of the diplococcus, since a high concentration of the serum is more effective than a low one. But the mechanism of test-tube bacteriolysis and of intradural bactericidal effect may be and doubtless are widely different. Our knowledge of the manner of *intra vitam* disposal of the diplococcus after serum injection is very defective; but an important factor is doubtless the intracellular, phagocytic digestion for which we have evidence derived from microscopical examination of the cerebro-spinal exudate. While in active stages of the infection the intracellular diplococci present sharp outlines and appear vigorous, and the extracellular microorganisms are well preserved, after the serum injection the diplococci within cells have lost sharpness of outline, stain indifferently, and strike one as degenerated, and those without cells are much reduced in numbers and staining power. Possibly it is this active and apparently accelerated intracellular digestion which prevents an increase in toxic effects following the serum injections such as might otherwise occur from the more rapidly liberated endotoxin. That the antiserum possesses certain direct antitoxic properties, which also tend to di-

minish the dangers of endotoxin-intoxication, would seem to be indicated by its power to neutralize *intra vitam* the toxic effects of an autolysate of the diplococcus.

In order to avoid unnecessary repetition in preparing this discussion, some of the above propositions have been stated in a manner that might readily convey the impression that we regard it evident and established that the antiserum has proven its usefulness as a therapeutic agent in epidemic meningitis. The facts of our belief, at the present time, are quite otherwise. No one could be less convinced of the final fact of its value than we are. On the other hand, we believe that the data at hand warrant a wider trial of the antiserum, particularly as no other and better means of combating the disease is available. We think, however, that it is unjustifiable to employ the serum indiscriminately and without proper clinical and bacteriological controls. We shall be able, at the Rockefeller Institute, to supply a moderate amount of the antimeningitis serum for use under conditions of control which we shall prescribe.

ADDENDUM.

Since the completion of this paper reports of seventeen additional cases of epidemic meningitis in which the antiserum was employed have been received. The full histories of the cases will be published later, but summaries of them will be given here. Twelve of the cases were treated at the Municipal Hospital, Philadelphia, by Dr. Franklin Royer. A tabulation of them follows:

Case No.	Age of Patient in Years.	Day of First Serum Injection.	Total Amount in c.c. of Serum Injected.	Result.
I.	3	7	180	Died.
II.	4	9	90	Recovered.
III.	18	3	90	Died.
IV.	7	3	140	Recovered.
V.	3	4	220	Died.
VI.	10	2	90	Recovered.
VII.	8	6	30	Recovered.
VIII.	22	?	95	Recovered.
IX.	13	3	150	Recovered.
X.	10	8	90	Died.
XI.	10	4	150	Recovered.
XII.	13	3	120	Recovered.

Two babies, each a year old, were treated with the antiserum at Mt. Sinai Hospital, New York, by Dr. Heiman. One received the first injection of serum on the twenty-third day, 60 cubic centimeters were injected in all; it died. The other was injected on the fifteenth day, received 35 cubic centimeters of the serum and recovered.

Two children were treated with the serum at the Babies Hospital, New York, by Dr. Holt. One is eleven months old, was injected first on the forty-ninth day of illness and will probably die. The other is eighteen months old, was injected first on the twenty-third day of illness and is now convalescent.

An additional case, not included in the first series of cases, was treated at the Akron City Hospital. The child was six years old, was injected first on the sixth day of illness, received in all 160 cubic centimeters of antiserum and recovered.

STOMACH FEEDING IN MICE.[1]

By DR. LEWIS HART MARKS.

Assistant in the Bacteriological Department of the Royal Institute for Experimental Therapeutics, Frankfort a/M.

(*From the Royal Institute for Experimental Therapeutics, Frankfort a/M. Director, Geheimrat Prof. Dr. P. Ehrlich; Hygienic-Bacteriological Dept., Prof. Dr. M. Neisser.*)

To be able to feed mice absolutely quantitatively has long been the desire of many experimentors. Of the many methods which have been devised for this purpose only one, that of Ehrlich[2] (feeding with prepared cakes), still survives. This method depends, however, upon the hunger or eating capacity of the individual mouse. The procedure which we present herewith is both quick and most easily performed. Since the introduction of this method, in this Laboratory under the direction of Professor Max Neisser, nearly a year ago, it has been in constant use for many different purposes, all of which form the subject of following papers, with the exception of the lethal and maximum dose of several drugs, which we present herewith.

Method.—The tube employed (Fig. 1) was made for us by the firm of F. and M. Lautenschläger, of this city. It consists of a silk rubber tube with a syringe needle head, being six and one half centimeters long. The forward end is rounded, as in the human stomach tube. The needle head projects very little into the lumen of the tube. With reasonable care these tubes may be kept for a long time. It is advisable to wash out the lumen with alcohol after using, in order to prevent clogging. In order to pass the tube the mouse is held in the following position. An assistant grasps between the thumb and first finger of the left hand the loose skin as far down over the nose as possible, the thumb being in line with the right ear of the mouse. The tail is now grasped

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[2] *Deut. Med. Woch.*, 1891, xvii, 976.

with the right hand, the mouse drawn straight, then the tail is passed and held between the ring and little finger of the left hand (Fig. 2). The assistant now passes closed, with the right hand, a small thin bladed forcep, into the mouth, then allows the forcep to open, which movement forces the jaws apart. Care must be taken to have a forcep which does not open too widely (Fig. 3). The tube is now wet with water, grasped about its middle, by the operator, and passed downwards and a little to the side, until its length is lost in the digestive tract and mouth (Fig. 4). Force even of the slightest nature must never be employed. If the mouse is held correctly, and if the tube is placed correctly, only gentle guidance is necessary. The syringe holding the desired quantity is now quickly inserted into the needle head, and the piston forced down. The syringe is quickly removed, and after a little water or salt solution has been drawn in, again inserted into the needle head, and its contents injected. This last procedure washes out all the remaining fluid in both the syringe and tube, and insures the entrance into the stomach of the exact quantity. The tube is removed by one quick upward movement. Mice have not the power of vomiting and are therefore especially adapted for feeding purposes.

MAXIMUM AND LETHAL DOSES.

General Remarks.—We undertsand by the term "Maximum Dose" the largest dose which failed to kill one, from a great number of mice, between seventeen and twenty-five grams in weight. We do not pretend that it is the medicinal dose, or that it can be borne upon successive occasions at short intervals. However, from our experience with the substances mentioned below and with other substances, we can state that as a general rule, if a mouse bears a given dose, by this method, or by intraperitonel or subcutaneous injection, once it can bear the same quantity upon succeeding days.

Calomel.

Owing to its insolubility the giving of this drug, by this method, is not so satisfactory. We have employed it however in suspension, with the following results:

Lethal Dose	0.005 grm.
Maximum Dose	0.002 grm.

Dilute Hydrochloric Acid.

The finding of the lethal dose of hydrochloric acid was due to the fact that we desired to use it as a solvent for quinine, which follows:

Lethal Dose ..... 0.5 c.c. from a 6 per cent. solution.
Maximum Dose .. 0.5 c.c. from a 5 per cent. solution.

Quinine Hydrochlorate, in 3 Per Cent. Hydrochloric Acid.

Lethal Dose ..... 0.1 grm.
Maximum Dose ..... 0.08 grm.

Iodide of Potassium.

Lethal Dose ..... 0.03 grm.
Maximum Dose ..... 0.01 grm.

Sodium Salicylate.

Lethal Dose ..... 0.035 grm.
Maximum Dose ..... 0.02 grm.

Sodium Sulphite.

Lethal Dose ..... 0.2 grm.
Maximum Dose ..... 0.09 grm.

Sodium Sulphate.

Lethal Dose ..... 0.2 grm.
Maximum Dose ..... 0.08 grm.

Magnesium Sulphate.

Lethal Dose ..... 0.4 grm.
Maximum Dose ..... 0.2 grm.

Antipyrine.

Lethal Dose ..... 0.04 grm.
Maximum Dose ..... 0.01 grm.

Bichloride of Mercury.

Lethal Dose ..... 0.0007 grm.
Maximum Dose ..... 0.0004 grm.

Strychnine Nitrate.

Lethal Dose ..... 0.00005 grm.
Maximum Dose ..... 0.00003 grm.

Atoxyl.

Lethal Dose ..... 0.04 grm.
Maximum Dose ..... 0.02 grm.

Sodium Acetyl-paraamido-phenyl-arsenic Acid (Ehrlich).

Lethal Dose ..... 0.3 grm.
Maximum Dose ..... 0.1 grm.

FIG. 1.

FIG. 2.

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A DETAILED STUDY OF THE CHANGES OCCURRING IN THE PHYSIOLOGICAL DEGENERATION OF ACTINOSPHÆRIUM EICHORNI.*

By WILLIAM TRAVIS HOWARD,

of Cleveland.

(From the Zoölogical Institute, Munich.)

INTRODUCTION.

For a clear understanding of the changes to be described in the series of *Actinosphæria,* the study of which forms the basis of this communication, it is necessary to give a description of a normal *Actinosphærium* and a resumé of Richard Hertwig's work on the physiological degeneration of this animal.

In life, a normal *Actinosphærium* is a spherical cell with numerous radially projecting pseudopodia, regularly distributed over the surface, and each with a homogeneous axis fiber. The protoplasm is sharply divided into a coarsely vacuolated cortical layer, which contains the contractile vacuoles and through which the bases of the axis fibers of the pseudopodia run; and an inner, more finely vacuolated protoplasm, the medulla, in which lie the nuclei and the food vacuoles. The nuclei are situated chiefly in the peripheral layer of the medulla, just beneath the cortex; but a few nuclei are scattered in the deeper parts of the medulla. The number of nuclei varies with the size of the animal. According to R. Hertwig, the nuclei, under normal conditions, vary from 10 to 14 μ in diameter, and are surrounded by a distinct nuclear membrane, and contain nuclear sap and an achromatic linin framework, on which lie the nucleolar substance and the chromatin. He regards the nucleolar substance as identical with the material of which the nucleoli of the tissue cells of metazoa consists. According to Hertwig, in the simplest, but least common conditions, as

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Giant nuclear animals die in a few days, but before death one or more of these nuclei may be thrown off. In stained preparations, giant nuclei show a separation of the chromatin containing nuclear mass and the chromatin free portions; the latter form one or two round fluid-containing vesicular bodies which lie in the chromatin rosette.

Then the nucleoli increase in size, become vesicular, and take on a reticulated appearance. With the growth of the nucleolar bodies, the nuclear reticulum is pushed to the periphery and disappears. The remains of the chromatin rosette are condensed; if there are two nucleolar bodies, the chromatin lies between them, if only one, the chromatin mass is compressed at one side, giving the appearance of a signet-ring. Rarely, the chromatin rosette is absorbed during the growth of the nucleus, leaving the nucleolar body without a chromatin rosette. The nuclei may increase in size from the usual diameter of 10 to 14 μ to 70 or even 196 μ, and thus in a few days the nuclear mass may be increased by from 125 to 3,000 times. The fusion of nuclei plays no part in the production of giant nuclei. The chief cause is a growth of the nucleolar substance. Deep depression does not always end in giant nucleus formation; it occurs most often after depressions at the termination of which the *Actinosphæria* have been able to multiply for weeks and months. Hertwig regards the giant nucleus formation in *Actinosphærium* as the result of a series of changes which lead to the growth of the nuclei at the cost of the protoplasm. The growth of nuclei has, then, increased for so long, that finally their growth intensity renders a normal course of life processes impossible, and death results. The formation of giant nuclei would be favored, therefore, by excessive cell function.

3. Hyperplasia and hypertrophy of nuclei occurred in a depressed culture of *Actinosphæria,* containing a few animals with giant nuclei; most of the animals were of small or of medium size, with numerous pseudopodia and well defined cortex and medulla. These animals showed an enormously increased number of abnormally small nuclei (from one-half to three-fourths the normal size). Otherwise they had the usual structure of *Actinosphærium* nuclei. This condition coincided with a period of excessive feeding and

active multiplication, and during a whole month (April) the animals were characterized by the great number and the smallness of their nuclei.

This period was followed by one in which the nuclei were increased in size, with their chromatin divided into branching strands. These "hypertrophied" nuclei differ from the giant nuclei in their smaller size and in the absence of such great changes in the nuclear structure.

Besides the "hypertrophied" nuclei these *Actinosphæria* contain recently divided nuclei and nuclei of more or less normal size and structure. The "hypertrophied" nuclei are often oval in form and, in diameter, measure in the whole animal, from 21 to 35 μ, rarely 40 μ.

In some of these nuclei the chromatin is spread out over the whole nuclear network—hyperchromatic nuclei. In others, numerous chromatic nucleoli with short radiating processes lie on the reticulum. In the third type there are numerous sharply circumscribed vacuolated chromatic nucleoli. Transition stages are distinguishable, from nuclei with a few chromatic vesicles, to nuclei with a single large vacuolated nucleolus.

The nuclear framework varies from a fine webbed, delicate, granular network to one with coarse, homogeneous meshes. The nuclear meshwork is always sharply separated from the surrounding plasma by a membranelike thickening which is a further distinction between the "hypertrophied" and the "giant" nucleus.

Hertwig also describes chromatin-free nuclei, which usually contain a central achromatic nucleolus. The chromatin of these nuclei has degenerated and the nuclear framework becomes gradually shrunken.

The protoplasm of *Actinosphæria* with hypertrophied nuclei may be covered, in the living animal, with fat drops. In fixed and stained animals, it is purple or brown, owing to the presence of very finely divided chromatin. Chromidia are absent. Not infrequently, the nuclei are in sausage-like strings. Finally, it often happens that parts of such animals may become necrotic and be cast off as brown multinuclear masses.

Hertwig brings forward evidence to show that these "hyper-

trophied" nuclei may divide and may also play a part in the functions of the animal; they may also become reduced to small nuclei.

In these *Actinosphæria,* depressed by overfeeding over long periods of time, in cultures under observation for months, R. Hertwig has described a remarkable series of changes in structure, comprising shrinking, fusion and disappearance of pseudopodia, loss of distinction between cortex and medulla, chromidia and pigment formation, the presence of fat, the occurrence of areas of necrosis, the extrusion of nuclei, the degeneration of nuclei, as well as various modifications in the size, number and structure of the nuclei.

In these cultures there were periods of great depression, followed by intervals of regeneration of more or less great degree, as shown by return, more or less complete, to normal structure. Hertwig lays stress upon the fact that these various phenomena are associated with changes of the nuclear apparatus, the basis of which is "an increase of the nuclear substance at the expense of the protoplasm," a condition which occurs in connection with periods of excessive overfeeding and which must be referred to the increased capacity to assimilate food. Thus, both hyperplasia and hypertrophy of nuclei results from increased functional activity. According to Hertwig's ('04) view, the nuclear mass of a cell increases in amount by taking substances from the protoplasm, which has the power of splitting up substances suitable for the formation of secretions and which are taken up into the nucleus.

As long as the movement of substances from the protoplasm to the nucleus is in the ascendency, the existence of the nucleus is assured; while a preponderance in the passage of materials from the nuclei leads to a partial or total dissolution of these structures. The nuclear hypertrophy may be brought about by overgrowth or fusion of nuclei; and the increase of nucleolar substances is the chief factor in the enlargement of the nuclei.

It is evident that in these degenerative changes described by R. Hertwig in *Actinosphæria* depressed by overfeeding, the equilibrium between nuclear mass and protoplasm is markedly disturbed and that there must exist a struggle for ascendency between the two forces which under normal conditions work in coöperation.

This leads to his "Kernplasmarelation" theory, according to which, "for each cell there exists a definite size-relation of nuclear mass to cell mass, which may be represented by the formula K/P (nuclear mass divided by protoplasm mass)" ('03). As the variations of the "Kernplasmarelation" depend, above all, upon the assimilation capacity of the cell and upon cell division, he distinguishes a functional and a division growth of the nucleus. It is the functional growth of the nuclear mass which concerns us here. His studies on protozoa have led R. Hertwig ('03) to the conclusion that the "Kernplasmarelation" underlies not only periodic modifications brought about by the ordinary processes of life, but that under certain influences, it may experience more lasting changes.

These influences are: (1) uninterrupted function, (2) hunger, (3) change of temperature.

The marked effects of these influences upon the "Kernplasmarelation" Hertwig and his pupils have demonstrated by experiments not only upon *Actinosphærium, Paramœcium, Dileptus gigas* and *Frontonia,* but upon the egg cells of various metazoa.

Hertwig's view of the influence of temperature upon the "Kernplasmarelation," has been confirmed by the interesting work of H. Marcus ('06) on the fertilized eggs of *Strongylocentrotus lividus.* Marcus developed the eggs at 9° C., 19° C. and 22° C., and found that, in the blastula stage, the nuclei of those bred in the cold were distinctly larger in proportion to the protoplasm than of those bred at the higher temperatures.

Returning to *Actinosphærium,* it has been shown that the "Kernplasmarelation" is distinctly disturbed in hunger, in overfeeding and in encysting; in hunger by decrease in the protoplasm, in overfeeding by the functional increase of the nuclear mass; and in encysting by an increase on the part of the protoplasm of its property of dissolving nuclei.

SPECIAL PART.

The *Actinosphæria* upon which the following observations are based were taken by Professor Hertwig from his "B" culture at various times from February 4 until May 6, 1907.

Professor Hertwig furnishes me with the following notes of the culture, which was started in the fall of 1906 and continously

overfed. "From the fourteenth to the thirty-first day of January there was enormous multiplication and feeding. The animals stopped feeding from January 31 till February 3, when feeding was resumed for a day, then there was no more feeding until February 11. On February 16 the animals were feeding enormously and there was much plasmogamy. On February 19 the culture showed many small food-free animals. After this there was heavy feeding, with increase in the size of the animals and plasmogamy until March 3. On March 11 and 13 they were feeding less. Then until March 27 there was enormous feeding, with clumping. From March 28 till April 3 there was decreased feeding.

"On April 6 the animals were in marked depression. On the eighth and eleventh day of April, they were feeding heavily again. Then, there followed a period of heavy feeding which lasted until April 25. On April 27 the animals stopped feeding and there occurred a period of marked depression, during which many of the animals died. There was no encysting. From May 1 to May 4 there was intermittent feeding. The culture died out on May 6."

The work assigned me was the accurate and detailed study of the changes present in animals taken at various stages of the culture by Professor Hertwig. These animals, with the exception of a few which were received alive, were fixed with picro-acetic and stained with boraxcarmine by Professor Hertwig, and given to me mounted in clove oil.

Descriptions and sketches were first made of the whole animals, which were then embedded in paraffin and sections cut at 7.5 μ. The conditions to which they were submitted were kept as uniform as possible.

For most of the organisms, the carmine stain was followed by either weak Delafield's hæmatoxylin, or by Heidenhain's iron hæmatoxylin. On the whole, the most satisfactory results were obtained in sections very lightly stained with Delafield's hæmatoxylin.

The material is best presented, perhaps, in groups arranged according to the months, from February to May inclusive, in which the animals were taken from the culture. In February, animals

were taken on the fourth, ninth, eighteenth and twenty-sixth. During most of this time, the culture was in depression.

The first animal, received alive February 4, was of medium size, had a few very much thickened, blunt, short pseudopodia, and showed no demarcation between cortex and medulla, and no evidence of feeding. In stained preparations, studied both before and after sectioning, the cortex and medulla were fused, the spaces of the protoplasmic meshwork were small and thickened. The nuclei were very much increased in number, and somewhat in size (13.5 to 15 μ in diameter). The chromatin was in large irregular masses, usually connected by coarser or finer bands, forming rosettes and occasionally horse-shoe shaped forms. There were neither chromidia nor pigment. There was no evidence of nuclear fusion or of division. The second animal, which had on the fourth shown in the culture the same naked eye appearances of degeneration as the first, was killed on February 9. During this interval of five days it showed evidences of recovery (regeneration or restoration).

When killed it had short thick pseudopodia. In sections, the cortex was of normal width, well marked from the medulla, with a meshwork of normal size and appearance. The protoplasmic meshwork of the medulla was condensed and in the center of the animal it was thickened by the presence of a finely granular material. The nuclei were but slightly in excess of the normal number and were confined to the medulla. They were large—15 to 16.5 μ in diameter. The chromatic substance lay either in a single round or oval mass or in several round masses of unequal size, and near the center. The nuclear framework and nuclear membranes were distinct. Occasional degenerated nuclei without chromatin were seen. Some of the nucleoli were vacuolated. There was no fusion, division or overlapping of nuclei. A few scattered chromidia lay in the medulla, but there was absence of pigment. There was no evidence of feeding. This animal, compared with the first, showed distinct evidences of regeneration after a severe depression; it had developed pseudopodia, reëstablished its cortical layer, and reduced the number of its nuclei. It was abnormal, however, in having hyperplasia, hypertrophy and degeneration of nuclei.

On February 18 two animals were killed. They were both large, long, oval in shape, with a few short, thick, blunt pseudopodia. Sections of the larger of the two showed a fairly well-defined cortex of very irregular width, and a close, condensed alveolar meshwork, which was covered with fine granular plasma, and, at the extreme border, about the whole circumference, beset with fine yellowish brown pigment granules. The cortex contained no nuclei and no chromidia. The medulla, which contained two partly degenerated *Stentors,* was of a wide alveolar meshwork, which was covered, especially about the nuclei, with finely granular material and here and there with pigment granules. There were a moderate number of round, oval and irregular chromidia, lying, usually, about nuclei, and often connected with nuclear membranes.

The nuclei, which measured from 12 to 13.5 μ. in diameter, were considerably increased in number and were hyperchromatic. The chromatin was occasionally in round, solid, central masses, but in most nuclei it was scattered in large round or oval deeply staining masses, which were sometimes separated and sometimes connected by thinner branches of chromatic material. The nuclear meshwork was often obscured by these large masses. When visible, the nuclear meshwork was wide meshed and of uneven size. There was no vacuolization of nucleoli.

The other animal showed the same changes, except the chromidia were not so numerous. It contained no food material.

The last animal of this group was killed on February 26. It was actively feeding, of medium size, oval in outline, and without pseudopodia. The cortex was well defined from the medulla, and contained several large vacuoles. In the whole animal, the nuclei appeared very numerous and chromatin rich, while throughout the medulla great quantities of large and small, round, oval and irregularly shaped chromidial masses were present.

In sections the cortex was narrow and in places fused with the medulla. The meshwork was condensed, invaded by nuclei and beset with chromidia and a few pigment granules. The medullary meshwork was close set, the walls of the alveolar spaces thick and in many places crowded with chromidia. The latter appeared partly as fine masses, partly as large round, oval, or irregularly

triangular branched masses. Here and there, also, it was in coarse vacuolated fibrils. These chromidial masses were often grouped about the nuclei and many were directly continuous with the chromatic substance of the nuclei.

The nuclei, which varied in diameter from 12 to 13.5 μ, were decidedly increased in number and were intensely hyperchromatic. There were two general types of nuclei; one, the most numerous, with the chromatic substance in large irregularly shaped masses connected by bridges of the same substance, the other, with the chromatic material scattered in small round or oval, irregular masses. The chromatic material, or nucleoli, rarely lay in the centers of the nuclei. The nuclear framework was fine and granular. The nuclear membranes were in general distinct, but, about some nuclei, they were absent in whole or part. The evidence that the chromidia arose from the nuclear chromatin was convincing, for a direct continuity between masses of intranuclear chromatin and these extranuclear chromatin bodies could be traced.

The second group comprised a series of eleven animals taken from the culture on the first, third, fourth, sixth, seventh, tenth, eleventh and thirteenth of March.

The animals of the first and third of March were of large size, feeding, with well-defined cortical layers, and rather numerous hyperchomatic nuclei of normal size. The first contained no chromidia and but little pigment. As Professor Hertwig suggested, this animal probably marked recovery after depression.

The two animals taken on March 4 were of medium size, not feeding and showed marked signs of depression, as evidenced by narrowing and condensation of the cortex, marked chromidia and pigment formation, and hyperplasia and hypertrophy of nuclei.

In sections the narrowed and thickened cortex was studded with fine refractile, yellowish brown pigment granules; the alveolar meshwork of the medulla was thickened by the presence of a homogeneous plasma and contained great numbers of chromidia and pigment granules. The nuclei varied from 10.5 to 16.5 μ in diameter, and were situated in the cortex as well as in the medulla. The chromatin was usually in the form of large irregular rosettes. The nuclear framework was distinct. About some of the nuclei

the membranes were absent in whole or in part, with disintegration of the nuclear substance (see Fig. 14). Many nuclei touched at their borders, and overlapping of nuclei was frequent. Some of the large oval nuclei were evidently examples of fusion of two nuclei (see Fig. 3).

Chromidia were abundant and were usually grouped about the nuclei, and in many instances a direct continuity between them and nuclear chromatin could be traced (see Figs. 1 and 2). They occurred as small round or oval, large, irregular, vacuolated masses and as chromidial networks.

The organism was extremely rich in pigment, which was so thickly placed over one-fourth the body as completely to obscure the protoplasm. Over the rest of the animal, the pigment was in single, scattered or in clumped, granules. It was often in an intimate relation to the chromidia. In this animal pigment granules, identical in appearance with those in the plasma, were found in the nuclei. They were scattered over the nuclear meshwork near or on the chromatic substance, and were most often found in markedly hyperchromatic nuclei from which chromidia were projecting (see Figs. 1 and 2). The animal showed no evidence of feeding.

The three animals of March 6 are amongst the most interesting of the series. They all showed narrowing of the cortex, which was invaded by nuclei; there was hyperplasia, hypertrophy and hyperchromatism of the nuclei, marked chromidia and pigment formation, with condensation and thickening of the protoplasmic meshwork. In one of these animals, which showed the largest amount of chromidia of any of the series, the thickened, narrowed nucleus-containing cortex was beset with fine highly refractile yellowish brown pigment granules. The protoplasmic meshwork of the medulla was obscured in many areas by chromidia and pigment. The chromidia were enormously abundant and, frequently, were covered and surrounded by larger and smaller clumps of pigment granules (see Figs. 6 and 7). The nuclei, which were hypertrophied (from 13.5 to 15 μ in diameter), were remarkably hyperchromatic, and chromidia were pouring out from them in large quantities (see Figs. 4 and 7). Many nuclei contained pig-

ment granules. Overlapping of neighboring nuclei (Fig. 4) was frequent, but no examples of true fusion of nuclei were observed. There were a few dissolving nuclei, with the nuclear membrane partly or completely absent and the chromatic material in small clumps. The animal was not feeding.

The animal of March 7, also not feeding, showed the same changes described for those of March 6. The pigment was even more abundant, the granules being not only scattered over the protoplasmic meshwork and about the nuclei, but often occurring in large clumps, usually in a nest of disintegrating chromidia.

Many nuclei contained pigment granules. Vacuolization of the nucleoli was common. Two other forms of nuclear degeneration were noted, shrivelling of nuclei and disintegration of the nuclear membrane, in whole or in part, with the escape of the nuclear contents into the protoplasm. Several striking examples of nuclear fusion were observed (Fig. 3).

The animals of March 10 and 11 were of medium size, not feeding and contained no chromidia; only one showed pigment. There was nuclear hyperplasia, hypertrophy and hyperchromatism. The nuclei varied from 15 to 18μ in diameter. Touching, overlapping and apparent fusion of nuclei were common.

The last animal of the series, killed March 13, was comparatively small, and had a few short thick pseudopodia and a very thin and narrow meshed cortex. The nuclei were smaller and less numerous than in the other animals of this series. The nuclei were confined to the medulla and were not hyperchromatic. There were a moderate number of chromidia and no pigment. The animal was not feeding.

The third group consisted of a series of twelve animals killed on sixth, seventh, eighth, tenth, and eleventh of April. They were, with two exceptions, small.

Two animals were killed on April 6. The first was of medium size, contained no pigment and no food. The demarcation between medulla and cortex was distinct. The meshwork of the latter was wide and contained well marked vacuoles. The protoplasmic meshwork of the medulla was of normal size, except in the center, where it was condensed. The meshwork, especially in this central

area and about the nuclei, was covered with homogeneous or very finely granular achromatic material. A few small round or oval chromidia were about some of the nuclei. In the spaces of the protoplasmic meshwork, there were large numbers of free vacuolated nucleoli (Fig. 10), from ten to fifteen being counted in a single section.

The nuclei were markedly increased in number, but were remarkably small, measuring from 7.5 to 10.5 μ in diameter. They were often crowded together in clumps, of two, three, four or more, their membranes touching over a small area. Overlapping of nuclei was common (Figs. 8 and 9). There was no fusion of nuclei and no division figures were to be found. The nuclei varied markedly in their chromatin content, some being markedly hyper- and others markedly hypochromatic. In the former the chromatic material was usually in irregularly shaped branching rosettes and was often beset with one or more large or small vacuoles. In the latter, the chromatic substance was usually in small round or oval scattered masses, often near the nuclear membrane. In some of these hypochromatic nuclei, the chromatin lay partly within and partly without the nuclear membrane. In one such nucleus, the nuclear membrane was lacking for about one-fourth the circumference. Some nuclei contained no chromatin and others only a few fine particles.

A common change in the nucleoli was vacuolization, which affected small as well as large chromatin masses. In the large nucleoli, the vacuoles were large and ring-shaped, and frequently a number of smaller vacuoles were grouped about a large central vacuole. In many nuclei, large vacuolated nucleoli were separated from the achromatic substance and in various stages of extrusion. Some of these bodies lay just outside the nuclear membrane. It is possible that these appearances were mechanically produced by the microtome knife.

Undoubtedly the most common change by which the large vacuolated nucleoli were set free in the protoplasm was by the dissolution of the nuclear membrane and the achromatic nuclear material. All the steps of this process could be readily traced.

Another set of changes which led to the presence of large vac-

uolated masses in the protoplasm, was seen best in sections stained with iron hæmatoxylin. This change was characterized by shrivelling and condensation of the nuclei, with the accumulation of the chromatic material about the condensed nuclear membrane, and by hyaline degeneration and vacuolization of the achromatic nuclear material. The vacuolated body resulting from these changes could be distinguished from the typical, free lying, vacuolated nucleoli, by the presence in the former of a group of smaller vacuoles or of hyaline nuclear substance lying within the chromatic ring (see Figs. 17 and 18). In the typical vacuolated nucleoli, the vacuoles appeared as empty spaces in the chromatic substance. Fig. 18 shows an interesting example of hyaline degeneration of the nuclear framework.

The second animal showed the same changes described in the first. The only special difference between the two animals was the greater number of nuclei and the smaller number of free vacuolated nucleoli in the second.

This animal was infested with great numbers of small (8.25 to 11.25 μ in diameter) closely packed, hyperchromatic nuclei. They showed to a less marked degree the same changes described in the first. The animal showed no evidences of feeding.

On April 7, two medium sized animals which were fused over a portion of their cortices were killed. They both contained small food vacuoles, were without pseudopodia and had narrow but distinct cortices, the meshwork of which was small but not specially thickened. In sections they were nearly identical in structure. The protoplasmic meshwork of the medulla was relatively wide, except in the center where it was condensed. In general it was covered with finely granular, achromatic material. There was entire absence of both pigment and chromidia. The nuclei were very numerous and relatively small, varying from 8.25 to 11.25 μ in diameter. They were, for the most part, hyperchromatic, the chromatic material being usually in large masses or rosettes. Nuclei with finely divided chromatic material were uncommon. In most nuclei the chromatic material was vacuolated. Some showed the large vacuolated chromatic nucleoli described in the animals of April 6 (see Fig. 11). In general, the nuclear framework and

membranes were distinct. There were a number of nuclei in various stages of dissolution, with loss of the nuclear membrane and disintegration of the reticulum, as described in previous animals; such nuclei had vacuolated nucleoli, and this was evidently one of the processes leading to the presence of vacuolated nuclei in the plasma. Another change resulting in such free vacuolated nucleoli was the shrinkage and concentration of nuclei in association with hyaline change of the nuclear framework, as previously described. The result was the formation of hollow, spherical or bizarre shaped shells of deeply staining chromatic material, enclosing one or more large vacuolar spaces and sometimes studded with smaller vacuoles (see Figs. 19 to 25). One of these masses of vacuolated nucleolar material measured 25 μ in length (giant vacuolated nucleolus, see Fig. 25). A common shape for the smaller masses was that of a signet-ring. Some of these free vacuolated bodies were evidently due to the extrusion of a vacuolated nucleolus before disintegration of the rest of the nucleus.

The three animals of April 8 were small, but actively feeding. Two had a few short blunt pseudopodia. The cortex was narrow but well defined from the medulla. They contained no chromidia, pigment, or free vacuolated nucleoli. The nuclei were distinctly, though not greatly, increased in number, and were hyperchromatic. They varied from 9 to 13.5 μ in diameter. The nuclear chromatin was arranged either in large loosely formed rosettes or in small scattered masses. There were no evidences of nuclear division, fusion or dissolution.

On April 10, three more animals were killed. They were all actively feeding and had a few short, stout, blunt pseudopodia. Their protoplasm was of normal structure and contained neither chromidia nor pigment. The nuclei were distinctly over-numerous and were hyperchromatic. They varied widely in size (from 9 to 15 μ in diameter); the smaller type of nuclei outnumbered the large.

Of the animals killed on April 11, two agreed in all respects with those of the tenth. The third had a distinctly increased number of large (15 to 18 μ in diameter) hyperchromatic nuclei. The nucleoli were often vacuolated.

The last group consisted of ten animals which were killed between the first and the sixth of May.

Three animals killed May 1 were small, round, actively feeding, and had a few short twisted pseudopodia, a well defined and developed cortical layer containing large vacuoles. The medullary meshwork was of unequal size and beset with large and small chromidia, which often showed vacuolization. The over-numerous and vacuolated hyperchromatic nuclei varied from 10.5 to 13.5 μ in diameter. There was no evidence of fusion, division or dissolution of nuclei. Pigment was absent in two. In one animal which was especially rich in chromidia, the latter were usually covered and always surrounded by innumerable, very fine dust-like particles of chromatic material, which obscured the protoplasmic meshwork. This deeply staining "chromatin dust" was quite distinct from the highly refractile yellowish brown pigment granules, which were also present in small numbers.

The animals of May 2, 3 and 4 were in all respects similar to those of May 1.

The animal killed on May 5 was shrivelled and distorted in shape. The abnormally wide cortex contained large irregular vacuolar spaces, separated by a coarse protoplasmic framework. The outer layer of the cortex was covered over wide areas with a deeply staining homogeneous material—dissolved chromatin.

The relatively small medulla consisted of a very wide and irregular sized meshwork, the coarse strands of which were covered with finely granular achromatic material and with "chromatin dust." There was a considerable number of large and small chromidia, but no pigment. The nuclei were hyperchromatic, with a coarse reticulum and vacuolization of the nucleoli. The nuclei measured 12 μ in diameter.

The last animal of the group and of the study, for the culture died out on this day, was killed on May 6. It presented a very peculiar appearance. In the whole animal, the outlines were very irregular and no separation between cortex and medulla could be discerned. When stained with borax-carmine, the animal presented a granular non-staining outer layer and an inner irregularly shaped, granular diffusely staining mass, which formed the bulk of the organism. In sections, there was no distinction between cortex and medulla and the protoplasm was obscured by the presence of

dissolved chromatin and large vacuolated chromidia. Only an occasional nucleus could be found. One which was measured had a diameter of 10.5 μ.

The progress and interrelations of the various changes observed in these "depressed" *Actinosphæria,* the methods adopted by the animals to readjust themselves and the successive stages in the finally unsuccessful struggle can be, perhaps, presented best by contrasting the appearances met with in the various groups.

The culture sprung from *Actinosphæria* which had been caught at the beginning of winter. The animals at that time showed to a high degree the capacity for encysting, which was gradually lost, so that animals exposed to warmth no longer formed normal cysts. Some of the animals perished at this time from the formation of giant nuclei. My material sprang from animals which had survived this period.

The animals of February and March, after a prolonged and only occasionally interrupted period of overfeeding, showed profound depression, marked at times by distinct and often well marked signs of restoration. Their protoplasm was degenerated to a more or less marked degree. There was a variable amount of nuclear hyperplasia, hypertrophy and hyperchromatism. The animal of February 26 and most of those in March showed large amounts of chromidia and often pigmentation. Throughout, there was dissolution of nuclei, and, in the later animals, fusion and shrivelling of nuclei, and often vacuolization of nucleoli.

In the April group, after another period of heavy feeding, the animals were in almost all respects, except for some of the modes of nuclear degeneration, quite different from those of the previous series. The chief characteristics of the animals of April 6 and 7 were the great number of relatively small nuclei (from 7.5 to 10.5 μ and 8.25 to 11.5 μ in diameter) and the occurrence of shrinkage, hyaline degeneration, and dissolution of nuclei, with the vacuolization of nucleoli, rather than the formation of chromidia, as the modes of reduction of excessive nuclear substances.

That these degenerative methods of reduction of nuclear substances may be effective is shown by the great differences presented by the remaining animals of this group. With few exceptions they

were feeding actively; their nuclei, while usually over-numerous, were greatly reduced in number, and the general appearance of the animals was less abnormal. The nuclei were, however, both hypertrophied and hyperchromatic, due probably to the heavy over-feeding. In the last animal of the April group the nuclei reached a diameter of 18 μ.

From the eleventh to the twenty-fifth of April the culture fed heavily, and on the twenty-seventh the animals were again markedly depressed.

The animals taken on the first two days of May were feeding, the cortical layers were fairly well developed. The nuclei were of full size (10.5 to 13.5 μ in diameter), decidedly hyperchromatic and over-numerous. The nucleoli were often vacuolated, but dissolution of nuclei and free nucleoli were not seen. There were, however, enormous numbers of chromidia and much "chromatin dust." Some of the animals contained pigment.

The animals of May 3 and 4 were small, without food, and had relatively large nuclei and a somewhat smaller number of chromidia.

The animal on May 5 was shrivelled and distorted, with relatively little medulla, and the cortex contained a considerable amount of homogeneous dissolved chromatin. Otherwise it resembled those of the two previous days.

In the animal of May 6, the protoplasm was markedly degenerated and distorted, and but few nuclei could be recognized.

Reviewing the whole material, it is seen that, during the entire period covered by these observations, the animals showed not only a hyperplasia of nuclear substances, but evidences of profound changes in the structure of the protoplasm. It will be noted that typical giant nuclei were not present (the period for this having passed) and that the hypertrophied nuclei of this culture did not reach the extreme size obtained by those of Professor Hertwig's 1902 culture. I have designated as "hypertrophied nuclei" those nuclei which exceeded the extreme limit (14 μ) which he gives for normal nuclei. While there were times when the nuclear mass was reduced considerably and when the protoplasm approached its normal structure, organisms with normal nuclei in normal protoplasm were not observed; the "Kernplasmarelation" was never reëstablished, and complete recovery did not occur.

With the exception of giant nuclei, the necrosis of parts of protoplasm, fat and the transformation of all the nuclei to chromidia, all the nuclear and protoplasmic changes described by R. Hertwig ('04) in *Actinosphæria* depressed by overfeeding and prolonged culture, were observed in the series of animals described in the present communication. In regard to the absence of fat, it should be stated that most of the animals had been fixed and stained (and therefore treated with alcohol) before they were received by me.

In addition to confirming R. Hertwig's previous observations in most particulars, three new points were established in regard to *Actinosphæria* depressed by prolonged overfeeding, namely, the occurrence of pigment granules in hyperchromatic nuclei; hyaline degeneration of the nuclear reticulum leading to the formation of shrunken, vacuolated nuclei which in some respects resemble the free vacuolated nucleoli of R. Hertwig; and, finally, the fact that in *Actinosphæria* with large hyperchromatic nuclei, the reduction of nuclear mass is accomplished mainly by the extrusion of nucleolar substance and chromatin as chromidia (chromidiosis), while in animals with great numbers of relatively small nuclei, this is effected chiefly by shrinking, dissolution and various other degenerations of nuclei.

GENERAL PART.

It remains now to interpret these various nuclear and protoplasmic changes in the light of recent knowledge and to point out their relation to certain aspects of the general physiology and pathology of the cell.

In regard to the increase of the nuclear mass, it was both actual and relative. With R. Hertwig ('04) I can recognize three forms of increase of the nuclear mass, (1) the enlargement of single nuclei, (2) increase in the number of nuclei and (3) increase in the number and size of nuclei.

As previously stated, in the present series of *Actinosphæria* the time for the formation of giant nuclei was passed and the hypertrophied nuclei did not reach the size attained in Professor Hertwig's 1902 culture—21 to 35 μ. One possible explanation is that most of my measurements were made of nuclei in sections, which had been treated with xylol. Some of the nuclei exceeded 18 μ in

diameter. All of the animals, however, had an excess of nuclear material, in the shape of either a markedly excessive number of small or large nuclei or of a less marked excess of large nuclei. The protoplasm, whether of large or of small animals, always showed more or less well marked structural changes and was relatively small in proportion to the nuclear mass.

R. Hertwig has pointed out that this increase in the nuclear mass must be due to increased functional activity associated with the continuous overfeeding and the resulting overnutrition. He has suggested the same explanation for the large nuclei of carcinoma and sarcoma cells and of certain cells in inflammatory processes.

The nuclear hyperplasia in depressed *Actinosphæria* must, of course, be effected by mitosis, and it is not unlikely that the functional growth (hypertrophy) may produce in R. Hertwig's sense sufficient "Kernplasma tension" to start nuclear division. There is absolutely no evidence that the chromidia of depressed *Actinosphæria* ever give rise to new nuclei corresponding to the secondary nuclei of the Thalamorphora arising from the "Chromidialnetz."

R. Hertwig ('04) regards the occurrence of nuclear hyperplasia in depressed *Actinosphæria* as a good sign, for such animals may assimilate and multiply enormously, while most giant nuclear animals die. The division of the nuclear materials into multiple small masses would, he thinks, afford better conditions for carrying on the life processes.

The formation of chromidia and most, if not all, of the various forms of nuclear degeneration described in these animals, are to be looked upon as the results of the use of physiological means, for, to a considerable degree, the same processes occur in encysting *Actinosphæria,* in which the number of the nuclei is reduced normally to an astonishing extent. Chromidia may occur in normal animals and in the encysting process a great number of chromidia are extruded from the nuclei in the cysts, though most of the reduction in the number of nuclei occurring before encysting is accomplished by absorption of nuclei rather than by dissolution into chromidia. As in the processes leading up to encysting, so in these depressed animals, abnormally stocked with nuclear materials, the

protoplasm exerts its power of destroying the nuclei in order to reëstablish the " Kernplasmarelation."

I have stated already, that there is no evidence that the chromidia of depressed *Actinosphæria* possess a propagatory function in the sense of Schaudinn and Goldschmidt ('04), *i. e.,* they do not form new nuclei. According to Goldschmidt's ('04) interesting theory, these chromidia represent excessive somatic chromatin needed and used by the animals to assist in the active function which they are carrying on.

Against this view are the facts that the greatest number of chromidia are not present in the more actively feeding animals and that the chromidia break up into pigment. In favor of his theory, however, is the fact that, when the nuclei are large and relatively less numerous in feeding depressed *Actinosphæria,* chromidia are much more plentiful than in animals with great numbers of small nuclei. That normal *Actinosphæria* form chromidia during functional activity cannot be denied. It is possible that in depressed *Actinosphæria* the existence of two kinds of chromidia is to be recognized, one highly functioning, somatic in type, and the other formed in the efforts of the animal to reduce its surplus of nuclear substances and destined to be got rid of.

The formation of pigment from nuclear material is of great interest. R. Hertwig ('02) first suggested that the pigment of melano-sarcoma cells is formed from chromatin extruded from the nucleus in an effort of the cell to reduce its nuclear mass and thus to reorganize itself. Later ('04) he described the transformation of chromidia to pigment and recognized the possibility of the development of pigment from the nucleus, and called attention to the fact that the two agents (cellular activity and cold) which best effect relative enlargement of the nuclear mass also produce conditions most favorable for pigment formation. He referred, of course, to autochtonous iron free pigment.

R. Hertwig's pupil Roessle ('05) has traced, in the cells of melano-sarcoma, the wandering of nucleolar substance from nuclei over-rich in this material and its transformation into pigment in the protoplasm. Roessle suggested that the pigment formation of senile atrophy, probably partly physiological and partly patho-

logical, is produced in the same way as the pigment of *Actinosphærium* and showed that, as far as micro-chemical methods go, the pigments of *Actinosphærium,* melano-sarcoma and brown atrophy appear to be identical, and are iron free. Meirowitz-Grandenz ('07), studying pigment formation in the skin after exposure to the Finsen light, was able to trace the development of pigment from nucleolar substance, both in the nucleus and in the protoplasm. The same processes were evident in the *Actinosphæria* described by me.

Through the courtesy of my friend, Dr. H. Marcus, I have observed pigment granules in the nuclei of pigmented epidermal cells in his sections of Professor Brauer's *Hypogeophis rostratus* material.

The nuclear degenerations present in these *Actinosphæria* have much in common with those which occur in various pathological processes in the higher animals, and, as R. Hertwig has shown, bear a striking similarity to some of the nuclear degenerations described by Pianese in carcinoma and erroneously interpreted as parasites by various authors. This is especially true of the vacuolization of nucleoli and the hyaline degeneration of nuclei met with in the present series of animals.

The condition of the protoplasm in many of these overfed *Actinosphæria* was as abnormal as that of the nuclei. There was loss or degeneration of pseudopodia, fusion of cortex and medulla, loss of vacuoles, condensation and shrivelling of the protoplasmic meshwork, and, in R. Hertwig's previous series, necrosis of protoplasm and the presence of fat. To what causes are these changes to be assigned? In the present state of our knowledge it is impossible to explain them satisfactorily. It seems, probable, however, that the exhaustion of overwork in digestion and in breaking down nuclei, the loss of materials to the ever-growing nuclei and the intoxication from retained metabolic products are among the factors concerned.

In conclusion, I take this opportunity to express my heartiest thanks to Professor Hertwig, not only for his material, his supervision and many suggestions in this work, but for many kindnesses extended to me in his institute.

MUNICH, May 22, 1907.

EXPLANATION OF PLATES XVI–XIX.

The drawings were made with the aid of a camera lucida, under Leitz 1/12 oil immersion objective, ocular 3, tube length 160 mm.

FIGS. 1 and 2. Hyperchromatic nuclei giving off chromidia, containing pigment granules, and surrounded by chromidia and pigment. Animal of March 4.

FIG. 3. Hyperchromatic nuclei; fusion of two nuclei. Animal of March 7.

FIG. 4. Small portion of periphery of animal of March 6. Note fusion of cortex and medulla, overlapping of nuclei, chromidia and pigment.

FIG. 5. Large hyperchromatic nucleus, probably the result of fusion of two nuclei. Animal of March 3.

FIG. 6. Condensation of protoplasm, intense chromidiosis, with large central clump of pigment granules.

FIG. 7. A large nucleus surrounded by chromidia and pigment granules.

FIG. 8. Small area of medulla of animal of April 6, showing marked nuclear hyperplasia. Note that the nuclei are small and touch at their borders.

FIG. 9. Overlapping of two small nuclei. Animal of April 6.

FIG. 10. A free vacuolated nucleolus between two small nuclei. Animal of April 6.

FIGS. 11 and 12. Nuclei with vacuolated nucleoli. Animals of April 6 and 7.

FIGS. 13 and 14. Stages of dissolution of nucleoli. Animals of March 4 and April 7.

FIGS. 15 and 16. Shrunken nuclei. Animals of March 4 and April 6.

FIGS. 17 and 18. Hyaline degeneration and vacuolization of nuclei. Animals of April 6 and 7.

FIGS. 19 to 25. Types of vacuolated nucleoli lying free in the protoplasm. Animal of April 7.

All the photographs (Figs. 26–28) are taken at the same magnification = × 100.

FIG. 26. Photograph of section of normal *Actinosphærium*. Note the wide cortex and the number and distribution of the nuclei.

FIG. 27. Photograph of section of a depressed *Actinosphærium*. Note hyperplasia, hypertrophy and hyperchromatism of the nuclei, condensation of the protoplasmic meshwork and the presence of chromidia. Animal of March 6.

FIG. 28. Photograph of section of a depressed *Actinosphærium* during a period of active overfeeding. Note the numerous vacuoles and marked hyperplasia and hyperchromatism of the nuclei. Animal of April 7.

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Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Fig. 8.

Fig. 9.

Fig. 10.

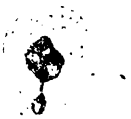

Fig. 11.

Fig. 12.

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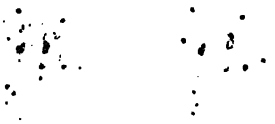

Fig. 15.

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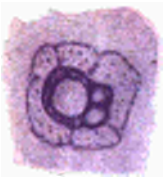

Fig. 19.

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Fig. 21.

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Fig. 22.

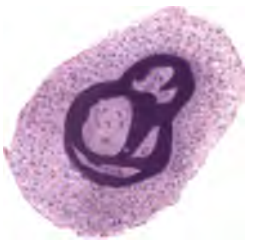

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Fig. 25.

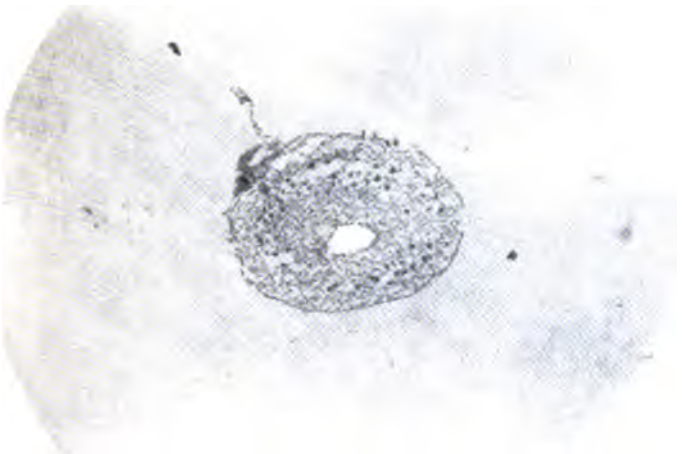
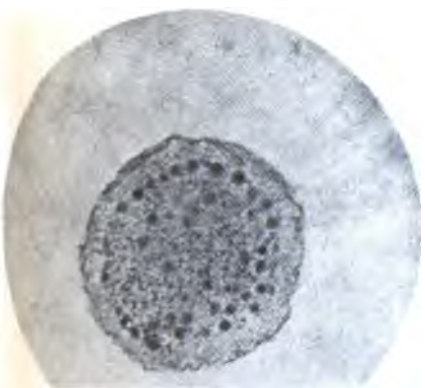


Fig. 26.



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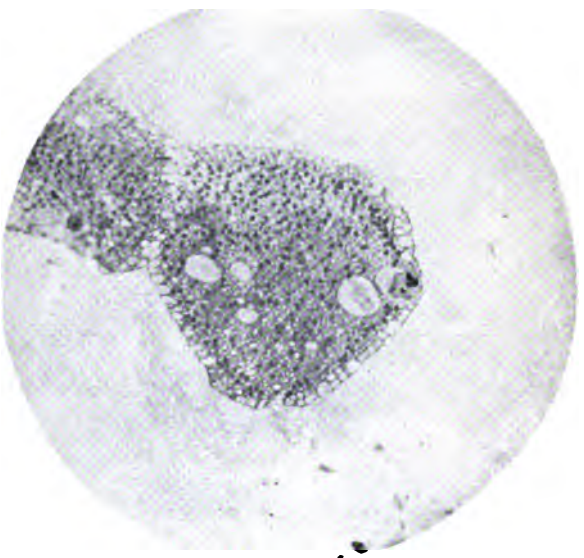

Fig. 28.

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THE OPHTHALMO-TUBERCULIN REACTION IN CATTLE.*

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von Pirquet[1] has recently made use of the local reactions sometimes elicited in individuals affected with infectious diseases when the toxin from the microörganism causing the infection is injected into the body. He has applied the phenomenon to tubercular infections and has found that when tuberculin is introduced into the dermis that a local reaction results in tuberculous subjects and no reaction of any consequence in the non-tubercular. This local cutaneous reaction lasts at least eight days. von Pirquet has applied this test in infants and children and finds that it is of diagnostic value especially in the beginning of the disease.

Vallée[2] has applied the cutaneous tuberculin test to cattle, horses and guinea-pigs. He finds that the reaction is positive in the majority of tuberculous animals and conversely does not occur in normal animals. It is not within the province of this paper to consider in detail the cutaneous reaction for tuberculosis. This subject will form the basis of a later communication.

Calmette[3] has made use of the foregoing principle, but has modified the method of applying the test. He instills the tuberculin into the eye instead of into the skin. He finds in cases of tuberculosis, after six to sixteen hours, that the conjunctiva, particularly

* Received for publication November 1, 1907.

[1] von Pirquet, *Ber. klin. Woch.*, 1907, xliv, 644.

[2] Vallée, *Compt. rend. de l'Academie des sciences*, 1907, cxliv, 1383.

[3] Calmette, *Presse médicale*, 1907, xv, 388, 443.

the palpebral conjunctiva, becomes markedly congested, that there is lachrymosis and finally the whole conjunctiva is covered with a serofibrinous exudate. In the non-tubercular there may be a slight conjunctivitis, but usually there is no reaction whatever.

Olmer and Terras[4] have found that the ophthalmo-tuberculin test in man is more accurate than the cutaneous test. They obtained some conflicting results, however, as in a few cases there was a reaction in non-tuberculous individuals whereas in a small proportion of those clinically showing tuberculosis there was no reaction.

In the light of the recent results which have been obtained by the use of tuberculin in the eye for the diagnosis of tuberculosis in infants and to a limited extent in adults, it has seemed possible that the ophthalmo-tuberculin test may be applied successfully to cattle. It is a fact, disputed by some, that bovine tuberculosis can be transmitted to man and especially to infants. This transmission takes place through the milk of tuberculous dairy cattle. It is important that an easily applied and accurate means for the diagnosis of tuberculosis in cattle be available in order that dairy herds be kept free of tubercular animals. Animals showing the least suggestion of tubercular infection should be isolated and if the diagnosis proves correct destroyed. It must be borne in mind that the tuberculin test as it is ordinarily made has many objectionable features.

With a view of investigating the efficiency of the ophthalmo-tuberculin reaction in cattle we undertook a series of experiments.

DESCRIPTION OF EXPERIMENTS.

Cattle.—The cattle used in these tests were divided into three classes as follows: Class A, five animals which reacted to the tuberculin test in February, 1907; Class B, five animals which reacted to the tuberculin test in September, 1907; Class C, twenty animals which were used as *control* and to which the tuberculin test was applied in November, 1906, no reactions resulting.

Class A.—These animals were fancy cattle and appeared to be in the best of condition with the exception of No. 2A, which was beginning to show clinical signs of tuberculosis.

[4] Olmer and Terras, *Presse médicale*, 1907, xv, 593.

Class B.—These five animals were from a herd of thirty-four dairy cattle, twenty-seven of which reacted to the tuberculin test. They are in the advanced stages of tuberculosis. *Bacillus tuberculosis* was found in the fæces of No. 2B.

Class C.—These animals were from a large dairy herd, nineteen of which had been repeatedly tested with tuberculin and had shown no reaction. Cow No. 2C is a two-year-old Jersey heifer and has a temperature (103°–104° F.) which is continually too high to be made use of in a usual tuberculin test. This cow will be tested as soon as possible.

TUBERCULAR CATTLE.

Class A.		*Class B.*	
1A.	Polled Angus	1B.	Jersey
2A.	Jersey	2B.	Jersey
3A.	Short Horn	3B.	Jersey
4A.	Short Horn	4B.	Jersey
5A.	Short Horn	5B.	Jersey

NON-TUBERCULAR CATTLE. CONTROLS.

Class C.		*Class C.*	
1C.	Grade Short Horn	11C.	Guernsey
2C.	Jersey (heifer)	12C.	Guernsey
3C.	Grade Short Horn	13C.	Jersey
4C.	Grade Short Horn	14C.	Jersey
5C.	Grade Short Horn	15C.	Red Polled
6C.	Dutch Belt	16C.	Red Polled
7C.	Jersey	17C.	Grade Red Polled
8C.	Jersey	18C.	Red Polled
9C.	Jersey	19C.	Grade Holstein
10C.	Jersey	20C.	Red Polled

Technique.—The method first tried was the administration of the tuberculin as prepared by Calmette. A one per cent. solution was made by dissolving the precipitate, obtained by treating tuberculin with absolute alcohol, in sterile water. The solution was sterilized by steam at 100° C. One-tenth (0.1) to one-fifth (0.2) cubic centimeter of the above solution was placed in the conjunctival sac of each cow. No results were obtained by the use of this method. There is a possibility that the amounts used were too small.

The tuberculin which proved to give the most successful results was procured from the Bureau of Animal Industry, United States Department of Agriculture. It was used full strength and twenty-

five-hundredths (.25 c.c.) of a cubic centimeter was placed in the conjunctival sac of each cow with a sterile eye dropper. The right eye was used, its condition being noted and compared with the left at the time of injection. Accurate data were recorded in regard to the temperature before and after the instillation of the tuberculin into the eye.

Reaction.—In the right eye, beginning from six to eight hours after the instillation of the tuberculin (.25 c.c.) the lids appear slightly swollen. Photophobia and lachrymosis are present. Within from sixteen to eighteen hours there appears upon the conjunctiva, a thin layer of whitish gray membrane. This is particularly marked on the bulbar conjunctiva covering the sclera and on the membrana nictitans which is reddened. Thin films of this membrane are constantly being washed down over the cornea, where they appear as movable opacities, passing downward to the lower lid. The eye-lashes are agglutinated with greyish yellow exudate. The episcleral blood vessels are dilated. From the inner canthus is a discharge of straw-colored exudate, which dries to form crusts of dried exudate below the inner canthus. The left eye is perfectly normal.

Statement of Results.—The reaction above described was found in all the tubercular cattle, *i. e.,* Classes A and B, and a slight reaction in Cow 2C. It will be recalled that 2C had not been tested with tuberculin regularly on account of her continued high temperature. The fibrinous exudate in this case was very slight. Class A showed the most profuse exudate and the most typical reaction. These cattle had not been tested with tuberculin since February, 1907. This point should be noted: Class B showed the reaction, but not in so typical fashion as Class A. These cattle were tested with tuberculin only four weeks before the ophthalmo-tuberculin test was administered. This point should also be noted: All the control cattle, *i. e.,* Class C, with the exception of 2C, which showed a slight reaction, gave no reaction whatever. Cows 1C to 7C inclusive, were tested twice and showed no reactions. No rise in temperature or other constitutional disturbance was noted in any of the cattle.

CONCLUSIONS AND SUMMARY.

The conclusions to be deduced from this brief series of experiments are as follows:

1. The ophthalmo-tuberculin reaction is of some value for diagnosis of tuberculosis in cattle. A characteristic conjunctivitis with fibrinous exudation coming on from six to eight hours, reaching a maximum in from sixteen to twenty-four hours and disappearing in forty-eight hours, is noted in tubercular animals.

2. The reaction is more pronounced in those animals which have not been recently tested with tuberculin. With this reaction as with the usual tuberculin test one injection and reaction probably inhibit a second reaction during a period from six weeks to a year. The ordinary tuberculin test does not seem to interfere to any great extent with the ophthalmo-tuberculin test at least within four weeks. Class B, though recently tested, showed the reaction, although not to the extent of Class A, which was tested some time ago. The tuberculin test occasionally prevents absolutely a second reaction, and usually no second reaction occurs within six weeks to a year, as before stated.

3. In cattle recently tested with tuberculin by the subcutaneous method the ophthalmo-tuberculin reaction is only slightly reduced in its intensity. The ophthalmic test may possibly serve as a means of diagnosis of tuberculosis in cattle which have been tested with tuberculin by the ordinary method and will not react a second time, or where tuberculin has been injected into cattle in order that they may clear a second test.

Another possibility must not be overlooked in this connection. It is well known that animals which have a slight tubercular infection often show a very marked and typical reaction to the tuberculin test as it is usually made, and those animals which show themselves clinically to be in the advanced stages of tuberculosis often show only a very slight reaction. The animals in Class B are in the advanced stages of tuberculosis and this fact might perhaps account for the lower intensity of the reaction. The exact cause of the lower reaction in these cattle can only be determined by further experimentation.

4. No constitutional disturbance being noticed in any of the cattle

tested, that is, no rise in temperature, loss of appetite or falling off in the production of milk, it is evident that the instillation of tuberculin into the eye does not produce the general reaction which attends in some cases the subcutaneous injection of tuberculin, and is therefore decidedly advantageous. The exudate disappears and leaves the eye perfectly normal in forty-eight hours after injection.

5. If the ophthalmo-tuberculin test proves as efficacious as the foregoing experiments seem to indicate we have in it a comparatively rapid and easy means of diagnosing tuberculosis in cattle. Such being the case the method cannot fail to come into general use superseding the present laborious method of applying the test. Cattle can be injected and then inspected sixteen to twenty-four hours afterward.

There are many problems in connection with the reaction which must necessarily be studied. The following are a few of the propositions:

1. Is it possible to tell by the reaction how far the tubercular process has progressed in the body?

2. Is there any relationship between the intensity of the reaction and the number and severity of the tubercular lesions?

3. Will the test prove to be more accurate than the ordinary tuberculin test which is said to reveal all but four per cent. of the cases?

4. Will the test reveal tuberculosis after a subcutaneous tuberculin injection?

5. Will animals react a second time if the first tuberculin is placed in the eye and the second given by the ordinary subcutaneous method and conversely?

These and many more points must be thoroughly investigated before the efficiency of the test is proven.

The opththalmo-tuberculin test will be repeated on other cattle, and also on the cattle used in these experiments. Experiments are being made to determine the efficiency of the cutaneous tuberculin reaction in cattle. We wish in this paper to give only the preliminary findings.

AN INQUIRY INTO SOME MECHANICAL FACTORS IN THE PRODUCTION OF LYMPHOCYTOSIS.[1]

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The paper that follows deals with an attempt to separate out, and study, some of the factors which produce the clinical feature of lymphocytosis. The truth of Ehrlich's (1) doctrine that an absolute lymphocytosis is due, apart from changes in the productive activity of the lymphoid tissue, to a flushing out of the cells through increase in lymph-flow, though supported by clinical evidence, has never been proved. Indeed, there have been few attempts to come directly by experiment to the forces determining lymphocytosis; this, too, despite manifold labours to plot the fluctuation of the blood-content in lymphocytes caused by divers physiological and pathological conditions.

For the experiments here detailed the cell-output by way of the thoracic duct was utilized. Of late this path to the blood for lymphocytes has been held to be of comparatively little importance. The recognition of lymphoid centers in the bone-marrow, the study of the abundant lymphadenoid tissue of the digestive tract, the observations for a direct passage of the lymphocytes into the blood-vessels, and the realization that the lymph has important functions of its own, have tended to this conclusion, as have the many assertions that the lymph of the thoracic duct carries few lymphocytes in comparison with the blood's needs. Nowadays, as Delamere (2) says, we hold that "the lymphocytes are the casual guests of the lymph." They are supposed to be formed in the lymph-glands and the lymphadenoid tissues in general, the spleen, and the bone-marrow, with direct entrance through the vessel-walls as a frequent way by which they reach the blood.

[1] Aided by a grant from the Rockefeller Institute for Medical Research. Received for publication November 18, 1907.

Nevertheless, recent experimental evidence points to the thoracic duct as the chief way to the circulation for the lymphocyte. Biedl and v. Decastello (3), working on dogs, produced fistula of the thoracic duct, and found that the lymphocytes in the blood decreased between 18 per cent. and 62 per cent. in absolute number; suspecting accessory channels, they carefully ligated the lymphatics on both sides of the neck, and obtained in the one animal so treated a diminution in the lymphocytes of 79 per cent. Selinoff (4), in a study of the blood of 18 dogs with fistula of the thoracic duct, noted an even more marked decrease. Thus, for example, in two of his cases there were, respectively, 1,800 and 2,000 lymphocytes per cubic millimeter in the blood just prior to operation, and on the fifth day thereafter, in the first case, only 100 such cells, and on the seventh day, in the second case, only 200 such cells per cubic millimeter. He made certain by controls that these results could not be laid to the effects of the operation itself. Crescenzi (5) observed the blood after splenectomy and the establishment of a fistula of the thoracic duct in the same animals. He obtained a decrease in the lymphocytes of from four fifths to ten elevenths of their number. Parodi (6) following Crescenzi and Selinoff, came to the conclusion that, in dogs, fistula of the thoracic duct, with or without splenectomy, brings about a diminution in the quantity of lymphocytes in circulation. Unfortunately ne omits the figures supporting this. Yet those cited above seem convincing when one considers the direct anastomoses known to exist between the lymphatics and the blood-vessels (Lippi, Boddaert, Leaf (7)), and the undoubted migration of some lymph-cells directly through tissues into the blood-stream. True, the diminution that the figures represent is transitory; but this only emphasizes the presence of a compensatory mechanism that must mask, to an extent, the full effect of the fistula.

It has been objected that the number of cells furnished to the blood through the thoracic duct is quite inadequate to maintain the percentage of lymphocytes seen. But the important element in such calculations,—the term of existence in the circulation of the individual cell,—is not known. The number coursing through the thoracic duct (from 2,000 to 7,000 in the cubic millimeter of dog's

lymph—Winternitz (8)) may be quite adequate, as Biedl and v. Decastello are at pains to show, for the needs of the circulation.

Whether or not the thoracic duct furnish the majority of the lymphocytes to the blood, as above indicated, the system of which it is the outlet,—a more or less completely "closed" system with one principal duct,—is the part of the hæmatopoietic apparatus most accessible to direct investigation as regards variation in cell-output.

The cell-content of the lymph has been comparatively little observed, and this mostly before the discovery of the bone-marrow as a blood-forming organ, and hence before the study of the blood-cells in its modern sense. Following Virchow's (9) demonstration of the identity of the "small mononuclear" with certain elements found in lymph-glands, several observers showed these cells to be more numerous in the lymph coursing from a gland than in that coming to it (Heydfelder, Brucke, Frey (10)). Löwit (11), counting the elements from the thoracic duct of the rabbit, obtained a great increase in them by the administration of substances causing blood-leucocytosis,—a result which has since drawn some criticism. Winternitz took lymph from the vessels of the dog's thigh, following the injection of turpentine into the corresponding foot, and came to the conclusion that with inflammation of a part the cell-content of the lymph coming from it is increased, and the majority of the cells becomes one of polymorphonuclear neutrophiles. Goodall and Paton (12), during an investigation on digestive leukocytosis, made counts from several points in the lymphatic system, but with very irregular results, except for evidence that pointed to a sedimentation of the cells in the receptaculum chyli. Recently Forgeot (13) has examined the lymph of ruminants as it escaped from a thoracic duct fistula, with no constant findings, however, beyond that of a greater cell-content in the fluid from young individuals. There have been no adequate researches on the cell-content of the lymph under varying physiological conditions. Yet, needless to say, the quantity of cells in the lymph represents one side of the activity of the lymphadenoid tissue; and variations in this quantity, in addition to their value as an index to the state of that tissue, have a bearing on clinical lymphocytosis and lymphopænia, and, ultimately, on the meaning of the lymphocyte.

To obtain a count that represents the average number of elements in the lymph flowing at a certain time, one should obtain a thorough mixture of a considerable quantity of it. For, by reason of the inconstancy of lymph-flow, as tending to a sedimentation of cells, and the anatomical arrangement of the lymphatics, which prevents the mingling of successive portions of the fluid, it follows that the individual drops, as they come from the vessel, must differ much in cell-quantity. Nevertheless one finds that of the few authors who have interested themselves in the cell-content of the lymph, practically all have taken their counts from the single drop,—which accounts for much of the irregularity in their results. Those above cited did this, except Forgeot who, in his work on ruminants, utilized one quarter of a cubic centimeter,—an extremely small quantity, considering the large size of the animals on which he experimented. Dastre, Henri, and Stodel (14), in an investigation of the effect of peptone on the cells, allowed the fluid to collect in the ligated end of the thoracic duct, or in the subclavian vein, there mixing it "by light, inconstant pressure." But, in addition to awkwardness, this measure gives the opportunity for large error in successive counts. I have employed, for the work here reported, a means whereby could be utilized a quantity of lymph sufficient to insure a result representing the average cell-content of the lymph at that time. Since dogs were the animals used, and the thoracic duct the point of collection, several cubic centimeters were deemed necessary for each test, owing to the large lymph-flow (64 c.c. per kilo per diem, or, in a dog of 18 kilo, 4 c.c. in every 5 minutes,—Heidenhain (15)). The following technic was adopted:

Three cubic centimeters of lymph are allowed to flow into a tube that contains an equal quantity of a 4 per cent. solution of sodium citrate in 0.8 per cent. salt solution,—a mixture suggested by Wright (16) for the preservation of blood unclotted and with its elements intact.[2] The tubes for this purpose are 9 mm. in bore, and are graduated accurately to 3 c.c. and 6 c.c. By reason of the

[2] Wright recommends the use of 1 part of the sodium citrate solution to 5 parts of human blood. So little of the solution will not keep the dog's lymph from clotting. A mixture of the two in equal parts is just sufficient to serve the purpose.

narrow caliber it is easy to control an error in volume to within the limits of a single drop, and, with care in the preliminary introduction of the sodium citrate solution, to confine this error almost entirely to the amount of lymph added. But let us suppose in the combined bulk of 6 c.c. (or about 90 drops) the largest tolerable error,—that of one drop in the quantity of sodium citrate solution added, and of one drop in the total mixture. The extremes here possible are, 44 drops sodium citrate solution to 47 drops lymph, and 46 drops sodium citrate solution to 43 drops lymph; or 47/91 of lymph in the first and 43/89 in the second mixture, a variation from the supposed ratio (45/90) of slightly over 3 per cent., or an error of 150 cells in a count of 5,000. This may be neglected.

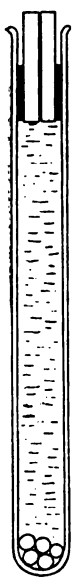

FIG. 1.

The lymph as it falls from the cannula into the sodium citrate solution is mixed with this by means of a fine wire, and, when the 3 cubic centimeters have been obtained, a few glass beads are introduced to aid in the distribution of the cells on shaking, and the tube stoppered preliminary to this. For stoppering a piece of glass rod with a flange of rubber is used, a capillary opening through the center of the rod permitting the escape of air so that the stopper may be pushed flush with the fluid. (*Vide* sketch in cross-section.) The closed tube is shaken for 5 minutes; a portion of its contents drawn into a "melangeur" with 1/100 its bulk of a saturated aqueous solution of methyl violet (5B); this in turn shaken for three minutes, and a count made as for blood. The lymph is thus counted in about 1/2 concentration (99 parts lymph to 101 parts diluting fluid). Leukocytes take the violet stain, whereas erythrocytes do not.

To test the method counts were made at intervals from the same tube of lymph-sodium-citrate mixture.

Thus at the end of seven hours the leukocyte-count coincides, practically, with that first taken. The cells are undergoing degenerative changes at that time, yet may be easily enumerated. But it is necessary, for this, that the tube be agitated at least once per hour. Otherwise, the cells sediment, cohere, and cannot be easily distributed again.

Dog.	Tube No.	Time between Counts.	R. b. c. per cmm. Lymph.		W. b. c per cmm. Lymph.	
			At First Count.	At Second Count.	At First Count.	At Second Count.
Jl	V	2 hours	not tested		3,880	4,240
El	XII	2½ hours	440	440	11,740	12,360
Nl	VII	2½ "	39,840	22,240	8,980	10,080
Ol	VI	3 hours	3,940	1,810	not tested	
Ml	V	3½ hours	5,080	3,720	3,300	3,660
Ll	II	4 hours	18,560	10,960	11,920	11,180
Gl	III	5 "	not tested		13,120	13,440
Kl	I	5 "	1,800	1,000	2,400	2,120
Ol	I	5 "	not tested		6,760	6,900
Pl	I	5 "	3,120	1,180	2,760	2,800
Jl	III	7 "	14,720	12,800	4,060	3,800
El	X	18 "	not tested		7,040	6,440

It is different with the erythrocytes. These seem to disappear rapidly in the mixing fluid, notwithstanding the fact that, to judge from the unchanged or slightly crenated shape of such corpuscles as remain, and the absence of shadows, this destruction is not due to osmotic changes. Since the lymph of nearly all dogs contains red cells, an idea of their quantity, granting them to be due to blood-contamination, is important in work having to do with the white cells of the lymph, since it furnishes an index to the number of leukocytes also brought in from the blood. But if, on the meeting of the lymph with the sodium citrate solution, many of the red cells go immediately to pieces, this index is destroyed; and one might have in the specimen many white cells from the blood without trace of the contamination, so far as red cells are concerned. This possible source of error was tested for as follows:

Dog Cl.—Blood taken during the experiment had 8,600,000 r.b.c. and 13,800 w.b.c. per cmm., of which last 70 per cent. proved to be polymorphonuclear neutrophiles, giving thus 9,660 such cells in the cmm. of blood, or about 1 to every 890 r.b.c. The lymph at this time contained on count from the sodium citrate mixture 1,545 w.b.c. and 5,685 r.b.c. per cmm. Calculating from the ratio existing in the blood, one should have 6 polymorphonuclear neutrophiles introduced with these red cells. A differential count of the lymph obtained at the time will test this supposition, since the normal lymph of the dog contains extremely few polymorphonuclear neutrophiles of its own (Delamere, Biedl and v. Decastello). As a matter of fact, in this instance the lymph showed out of 384 cells counted 2 polymorphonuclear neutrophiles, or 8 in the 1,545 w.b.c. of a cmm. So the number of red cells found in the lymph seemed to be practically that introduced from the blood. Several controls of this type gave the same result.

Despite this proof that the sodium citrate solution is, for *prompt* enumeration, a medium wherewith can be obtained an approximate estimate of the red cells, no lymph was admitted for the white count, of which the erythrocyte content was large enough to suggest that the accompanying leukocyte contamination might influence appreciably the results. The number of polymorphonuclear neutrophiles found in the lymphs used formed, as above shown, an additional indication of the amount of this contamination.

In the work here detailed the lymph's content in white cells is alone dealt with.

The effects of muscular exertion (struggle) on the cell-content were first observed. Adult dogs were employed. They were given 0.5 centigramme of morphia sulphate per kilo of body-weight (Nolf (17)) 1 hour before the operation, and chloroform when necessary during it. For from 24 to 48 hours prior to the operation no food was allowed the animals, though they were provided with water. The thoracic duct was bared in the usual way, a cannula introduced into it just above its entrance to the vein, and the entrance tied off together with such lymphatics from the neck as joined the thoracic duct, with the result that the fluid brought by the thoracic duct proper was alone collected. During this procedure very little blood, at most 3 to 4 cubic centimeters, was lost. The cannula used was of narrow bore, as recommended by Nolf, since the rapid flow through such a tube allows little opportunity for clotting. Nevertheless, in about half the experiments a delicate clot formed within the cannula in the course of some minutes, so that the occasional use of a fine hooked wire was required to keep the bore clean. No tubes of lymph were counted in which the least clotting appeared, nor were any used regarding which it seemed possible that clots in the cannula might have altered the gross cell-number. The presence or absence of clotting is mentioned in the report of the individual experiments.

It was first necessary to observe the variations in the lymph's cell-content under the circumstances above outlined and with the animal quiet, since these circumstances do not imply an absence of changes that might affect the cell-content. The shunting of the lymph from the body, following the opening of the thoracic duct,

produces marked alterations in the body-fluids (the blood, for example, concentrating, the lymph becoming less in amount and of different character). This might affect the lymph's cell-content. Furthermore, as the experiment progresses, the effect of the morphia wears off and chloroform must be pressed into service. Other unavoidable changes might be cited. The behavior of the cell-content under these influences must be reckoned with before one can proceed.

Accordingly, in animals carefully anæsthetized to a state of quiet, though not of complete muscular relaxation, a lymph-fistula was established, and specimens of lymph collected at short intervals during the next several hours. The time required to obtain each portion of three cubic centimeters was carefully noted as indicating the rate of lymph-flow at that period. Full records were also kept of all restlessness of the animal, of the incidents of anæsthesia, etc. When important, these are included in the description of the experiments.

Experiment I.—Mongrel collie; male; wt. 13 kilo. The ainmal was given no food for 48 hours previous to experiment. Throughout the time of lymph-collection it was quiet, except for occasional tremors in the limbs. Lymph very slightly opalescent; no clotting noted in cannula or tubes. Seven tubes were taken, and immediate estimate made of their content in white cells.

The dog at autopsy proved to have been sound, except for a chronic thickening of one segment of the tricuspid valve; no evidences of functional insufficiency of this valve.

The results are best expressed in the form of a chart. (Chart 1.)

Of the three curves on this chart one represents the rate of lymph-flow, a second the number of cells per cubic millimeter of lymph, and the third (which is the resultant of these two) the total cell-output in a given period.

It will be observed that throughout the course of the experiment the lymph-flow gradually but steadily lessened in rapidity, and hence in amount voided. This is, of course, no new finding (Lassar (18), Heidenhain and others). The number of cells per cubic centimeter of lymph, the "cell-concentration," as it will henceforth be termed, remained nearly constant, sinking slightly at the last count. It follows from these findings that the total cell-output underwent a marked gradual diminution.

To test these results two similar experiments were done.

Experiment II.—Collie; male; wt. 23 kilo. The animal was given no food for 48 hours previous to operation. Lymph began to escape from the thoracic duct (which had been ligated 1 minute before opening) 15 minutes prior to the collection of the first tube for cell-estimation. It was very slightly opalescent; no clotting was observed. Counts taken in each case immediately after collection. Eight tubes were obtained at half-hour intervals. Throughout, the animal was absolutely quiet. (See Chart 2.)

Dog killed and autopsied; it proved to have been quite healthy.

Here the same lessening of the lymph-flow is noted. The cell-concentration, markedly greater than in Experiment I, fluctuated

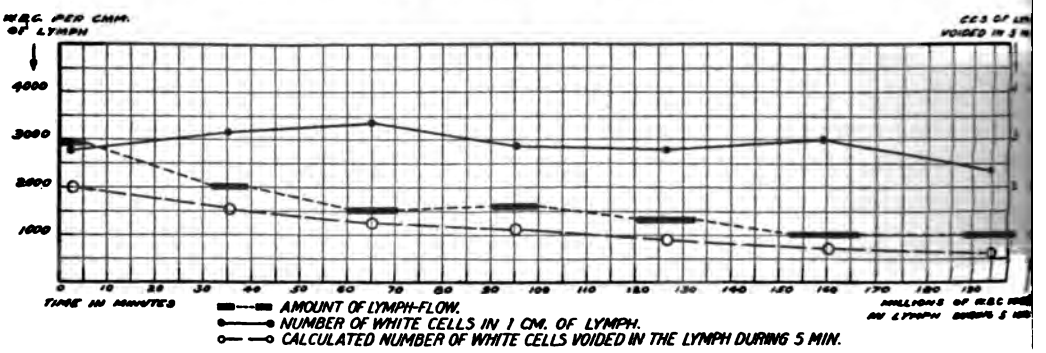

CHART I.

CHART I. The height above the base-line of the curve representing amount of lymph-flow indicates the number of cubic centimeters voided through the thoracic duct in a given time; and the black rectangles show the period required to collect the three cubic centimeters of lymph in each specimen. Thus the curve depicts in two ways the rapidity of lymph-flow.

proportionately, but in general remained constant during the first 2½ hours, after which it rose abruptly. The cell-output fell till toward the close of the experiment, when it regained nearly its former level.

Experiment III.—Skye-terrier; male; wt. 11 kilo. The fast before operation was of 24 hours duration, yet the lymph was quite chyliform throughout the period of observation. Clotting in the cannula necessitated several times the use of the hooked wire to avert blocking of the lymph-flow. The thoracic duct was ligated one half hour, and opened 15 minutes before the collection of the first tube for cell-estimation. The contents of the tubes were submitted to count in the order of their collection, but not till 3 to 4 hours after it, that is to say

at the close of the experiment proper. Seven tubes were collected at intervals in a period of 240 minutes. (See Chart 3.)

Animal normal, to judge from findings at autopsy.

A gradual drop in the rapidity of the lymph-flow occurred, similar to that in the other experiments, except for the presence of two transient fluctuations, apparently traceable to respiratory changes. The cell-concentration remained practically constant throughout the four hours, at the end of which it differed by only 100 cells per

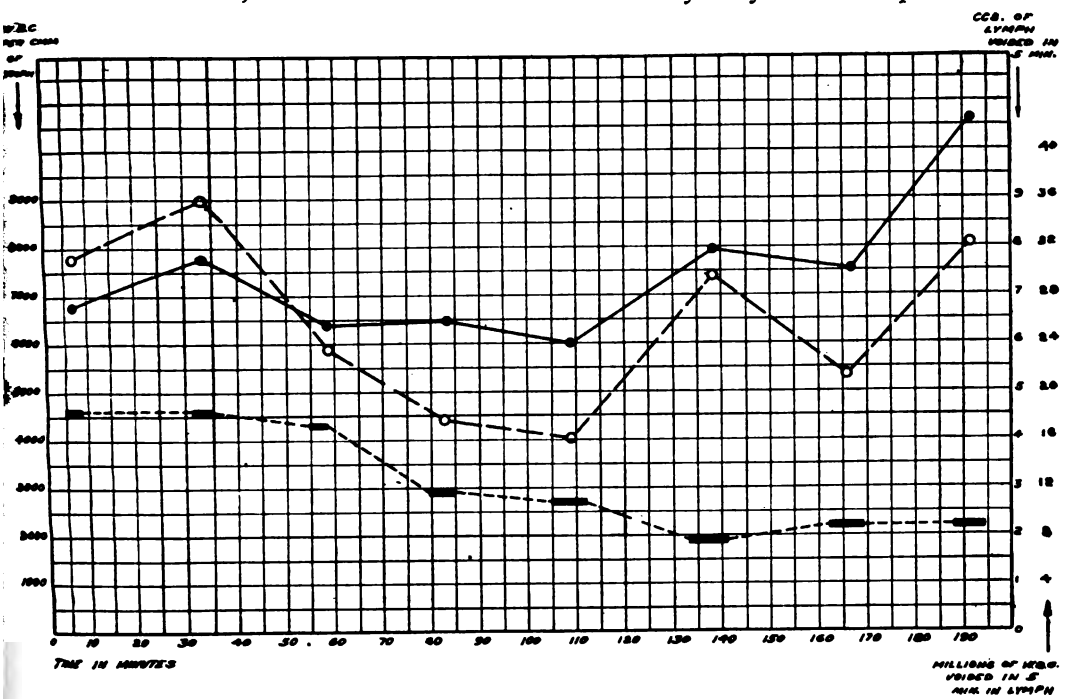

CHART 2.

cubic millimeter from that at the beginning. The total cell-output diminished, except during the fluctuations in lymph-flow above noted.

The results from the three animals form nearly a unit and are best discussed together. In all were observed:

1. A gradual decrease in the amount of lymph voided. This is no new finding.

2. A cell-concentration that varied little during the first 2½ hours of lymph-fistula. Quantitatively the variation accords with the degree of cell-concentration involved, being greatest in Experiment I, with its high cell-concentration (averaging 6,981 cells per cubic millimeter, from which there is a variation of 997 cells, or 17.6 per cent.) and least in Experiment II (in which the cell-concen-

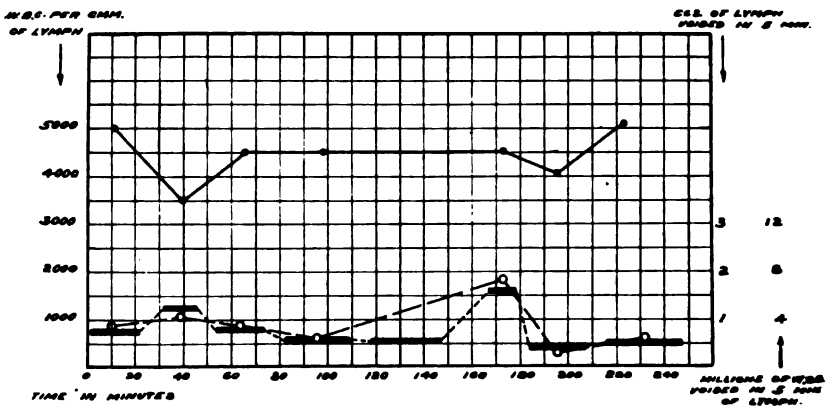

CHART 3.

tration averages 2,886 cells per cubic millimeter, and the variation is 506 cells, or 14.3 per cent.). In Experiment III the variation is 21.8 per cent.

So much for the cell-concentration during the first 2½ hours. Later, in one case it rose markedly, in another fell slightly, in the third remained unvaried. The only previous counts under conditions somewhat similar are those of Forgeot already referred to. The animals he employed (ruminants) were not anæsthetized, and his results show a variation, often of many thousand cells per cubic millimeter, in the counts from hour to hour. One understands this better on consideration of the small quantity of lymph he used in his estimations, the clotting that he frequently had to do with, and the absence of precaution to prevent struggle, which, as will later be shown, has a profound effect on the lymph's cell-concentration. Of fifteen animals on which he made observations, often over a period of more than 24 hours, the lymph in eight showed in general a tendency to lessened cell-concentration, in six, apart from large

transient variations, there was no change, and one exhibited an increase.

For our purposes it may be accepted that in the fasting dog anæsthetized with morphine and chloroform the cell-concentration of the lymph escaping from a fistula of the thoracic duct, is, during the first 2½ hours, fairly constant, when the animal is quiet and the lymph formation is suffered to take place undisturbed. The variation in cell-concentration during this period is not greater than 25 per cent.

3. The total cell-output, apart from transient fluctuations dependent on those in the lymph-output and cell-concentration, showed a decided tendency to lessen.

Here an interesting point presents itself for discussion: How is a constant cell-concentration maintained during 2½ hours under the conditions of a slowing stream of lymph, and a diminishing total cell-output? The explanation is not evident.

According to Ehrlich, the lymph-cells, on their maturation, are caught up by the lymph-stream and transported passively into the blood; or, as he puts it in another connection, "one is obliged to conclude that a lymphocytosis occurs, when, in response to an increased circulation of lymph in a greater or less extensive lymphatic region, more elements are mechanically forced from the lymph-glands." [3] Can one suppose that here the gradually lessening cell-output occurs because the lymph, through slowing, is rendered unable to force from the glands, and transport, its usual quota of cells? Or do the lymph-cells sediment along the course of the vessels in which they travel? Goodall and Paton, on the basis of counts from the receptaculum chyli, hold that some sedimentation in this reservoir is normal. Or do the lymph-glands, under the circumstances of the experiment, fail progressively in the maturation of cells? Any one of these happenings might explain the case.

Struggle, as will later be shown, increased promptly and markedly the cell-concentration of the lymph, although previously, under conditions of quiet, it had been lessening gradually. Thus it is

[3] The observations for an "active lymphocytosis" (Almkvist (19), Wolff and v. Torday (20), Proscher (21)) do not affect this conclusion, since they are concerned, not with lymphocytosis of the blood, but with the emigration of lymphocytes into the tissues and body-cavities.

shown that the glands are not lacking in cells fit for output. So the third hypothesis falls to the ground. One is left to explain in mechanical ways the constant cell-concentration in a lymph diminishing progressively in amount voided. One may suppose that the slowed current is not capable of transporting all of the many cells ready for it, and that, of those it picks up, some "sediment" on the way to the thoracic duct. The late rise in cell-content in Experiment II might, perhaps, be cited as an instance in which, despite these factors, the lymph became crowded with cells from the accumulation of those ready for it. In any event, the fact that the cell-concentration remains so long unchanged is surprising; one would expect to find immediately such variations as showed themselves only after several hours. Yet that the results are not (as might be supposed from Forgeot's work) examples of coincidence, is shown by the charts illustrating the effects of muscular exertion and of lymphagogue action. In these, despite varying cell-concentrations with varying physiological states, the same tendency to a constant cell-concentration is noted to occur hand in hand with a diminishing lymph-flow.

Discussion on the difference in average cell-output of the individuals will be reserved at this point.

With these results as a control, the effects of muscular exertion were taken up. It has been long known that this greatly accelerates the flow of lymph (Genersich, Lassar, Cohnheim (22)), but there have been no observations of its effect on the lymph's cell-content. A priori, on the theory that an increased output of lymphocytes is due, apart from special activity of the cell-forming tissues, to the flushing action of increased lymph-flow, one should find a transient increase in cells, traceable partly to those elements washed from the lymph-glands, and partly to those caught up from the channels by the swift current. But the existence of this increase, its amount, its duration, its effect on the blood, are all matters of conjecture.

In the experiments that follow, the animals were treated as those previously, except that they were at intervals made to struggle. Since morphia sufficed for the most part as anæsthetic, this was easily accomplished by giving a strong whiff of chloroform, or by tweaking the skin, or by an abrupt noise.

Experiment IV.—Irish setter; male; wt. 20 kilo; no food for 24 hours prior to operation. The thoracic duct was ligated, and a cannula introduced into it 5 minutes before the collection of lymph-specimens began. The lymph was slightly opalescent at first, later it was clear and yellowish; no clotting in cannula or tubes. The periods of struggle are noted on the chart. The cell-counts were made in the order in which the tubes were obtained, and between three and four hours after the collection of each one.

No autopsy done.

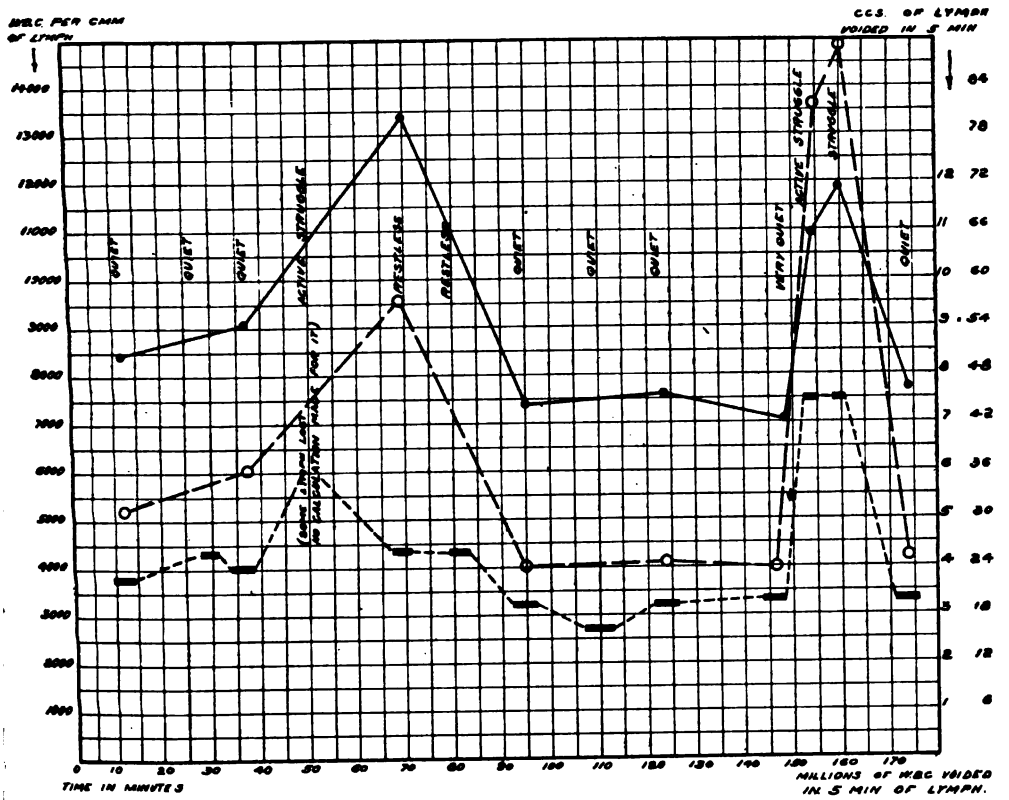

CHART 4.

In this instance struggle was twice induced. Each time the lymph-flow quickened abruptly and considerably, but with the return of quiet sank to below its former level. Each time, too, the cell-concentration became much greater. Thus the cell-output as a whole was multiplied. Indeed, it was necessary in this, and in the succeeding charts, to flatten the curve representing the cell-output.

With the return of quiet the cell-concentration and cell-output sank to slightly below their level previous to the exertion.

The next experiment was similar, but the lymph-specimens were collected at very short intervals.

Experiment V.—Bull-terrier; male; wt. 18 kilo; no food for 24 hours before operation. The duct was ligated, and opened, 4 minutes before the beginning of lymph-collection. Lymph at first slightly opalescent, yellowish and clear toward close; no clotting in tubes or cannula. The cell-estimations were made in the order of tube-collection, and 2 to 3 hours following this.

Animal at autopsy proved to have been sound. A tape-worm and several round-worms were found in the intestine.

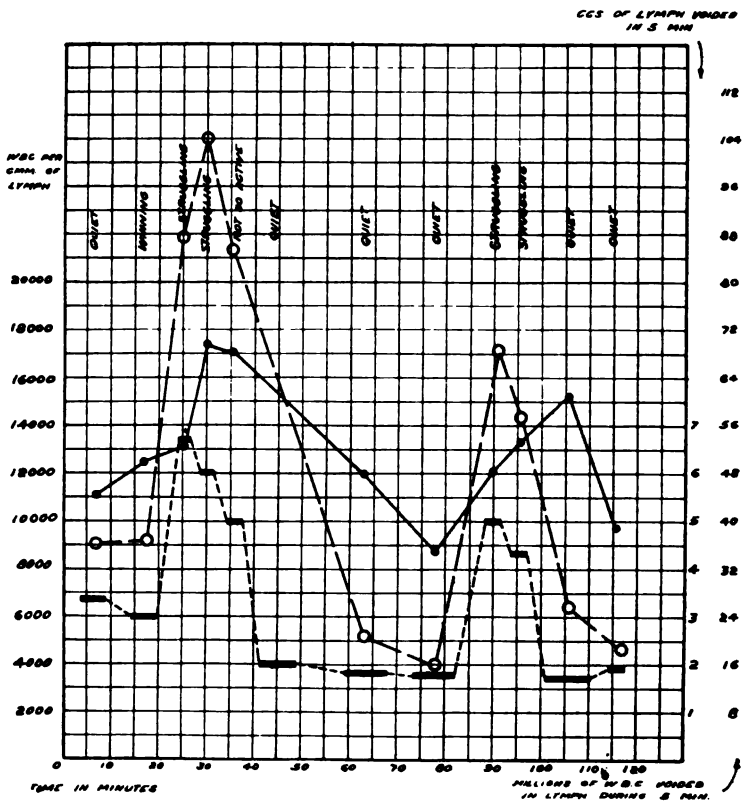

CHART 5.

The findings corroborate those of Experiment IV. Further, it is apparent that the greatest cell-concentration was not coincident

with the beginning of struggle, nor with the greatest lymph-flow, but came later. Indeed, that induced by the second struggle appeared after exertion had ceased. This second struggle did not bring out such a flow of lymph or mass of cells as did the first (which was similar in intensity); and, following both, the lymph-output, cell-concentration, and cell-output, all sank to below their level previous to the exertion.

In the next experiment the struggle was purposely made very long.

Experiment VI.—Spaniel; male; wt. 11 kilo; no food for 24 hours before operation. The duct was opened 10 minutes previous to the experiment proper, after it had undergone 5 minutes ligation. With whiffs of chloroform and tweakings of the skin a continuous struggle lasting 35 minutes was maintained. The lymph was slightly chyliform throughout; no clotting in tubes or cannula. Cell-counts were made in the order of tube-collection and between 2½ and 4 hours after this. (See Chart 6.)

At autopsy several tape-worms were found in the intestines.

This chart, while in general like the others, shows that the increase in cell-output during struggle is neither transient nor small. It endured so that at the end of the 35 minutes exertion there were being emptied from the thoracic duct 1½ times as many cells in each 5 minutes as during the preceding quiet. In the 35 minutes of muscular activity 48 cubic centimeters of lymph, containing an average of 5,100 white cells per cubic millimeter, were voided, as compared with 21 cubic centimeters of lymph, containing 3,100 white cells per cubic millimeter, in the 35 minutes just previous, or slightly over twice as much lymph, and, in sum, nearly four times as many cells as when the animal was quiet. Immediately following struggle there was a great fall in the total cell-output, and during the next 50 minutes it held to a low level.

In this series of observations the effects of five struggles were noted, and, during the work on lymphagogue action (*q. v.*), the effects of three more. They agree in these results:

(*a*) Struggle causes the cell-concentration of the lymph to become much greater. An attempt was made to test the parallelism of this increase with that observed in the lymph-flow, by collecting specimens at short intervals of time. This was done in Experiments V, VI, and during the struggle in Experiment VIII. The

charts of these prove that the maximum cell-concentration appears after considerable struggle-lymph has been voided, and at a time when the rapidity of lymph-flow is lessening. In one instance it was present in the slowly flowing lymph obtained on the return of quiet. These facts bear on the problem of whether the

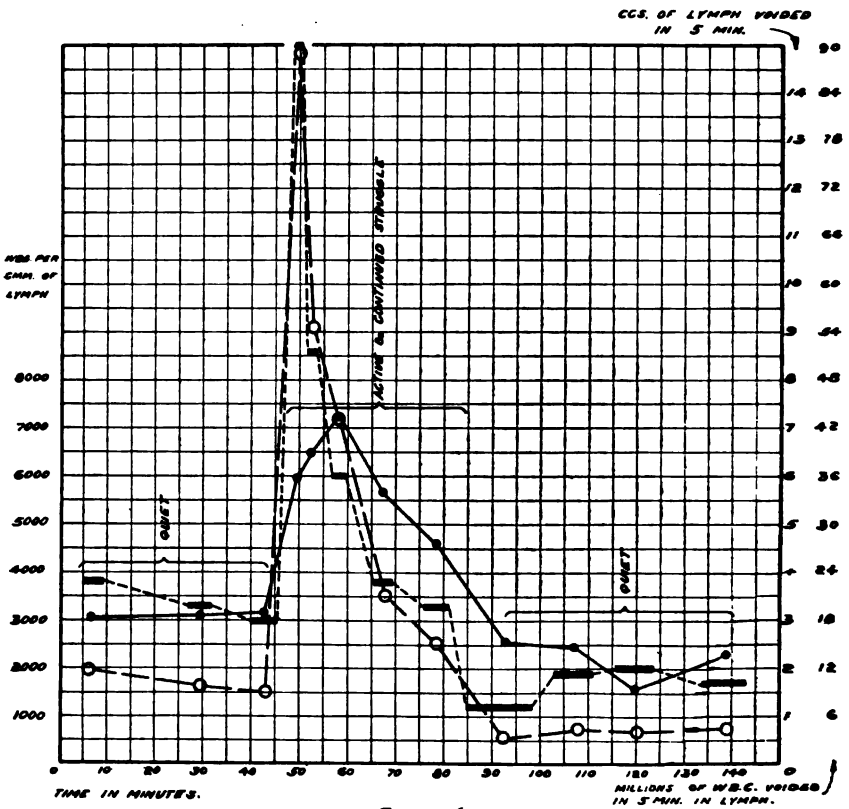

CHART 6.

cell-increase is due wholly to the flushing out of cells from the receptaculum chyli. Since the receptaculum is close to the opening of the thoracic duct, the cells flushed from it would appear, owing to the small size of the reservoir,[4] in the first few cubic centimeters

[4] I have made notes on the size of the receptaculum in several freshly killed dogs. The size varies widely, as does the shape of the reservoir, which indeed may not be present as such (Jussifow (25), and others). The walls of the receptaculum normally are held nearly apposed, so that the content is slight; but an impediment in the thoracic duct causes almost immediate dilatation with the accumulation of 1 to 4 c.c. of fluid.

voided during struggle; and, following this evacuation, the cell-increase would disappear quickly. But, in reality, the maximum cell-output was not infrequently delayed in its arrival (Experiment IV, second struggle; Experiment V, first struggle), and the maximum cell-concentration practically always was. In addition, the cell-increase is not transient (see Experiment VI) as would be the case were it traceable wholly to elements sedimented in the receptaculum.

Even granted that the first addition in cells comes from the receptaculum, whence is derived the later addition? Recent evidence (MacCallum (23), Buxton and Torrey (24)) speaks against v. Recklinghausen's view that the peritoneal cavity opens by direct channels into the lymphatics; and the quantity of free cells of this cavity is, normally, very small. The bulk of the increase is doubtless derived from the further lymphatic ramifications, in particular from the lymph-glands and other centers of lymph-cell formation. That the increased cell-concentration may persist for a short while after quiet has been restored is not surprising, since a host of cells, started from peripheral regions on the journey to the thoracic duct, need not arrive there until several minutes after the cause that got them under way had ceased to act.

(*b*) Struggle causes the cell-output by way of the lymph to become much greater. This effect persists throughout a struggle of considerable length.

In explanation of this phenomenon of increased cell-output it must be remembered that the lymph-region drained by the thoracic duct during struggle is larger than that during quiet. During the latter state, according to Starling (26), the fluid that arrives in the duct is derived, practically in toto, from the abdominal viscera. But muscular movement immediately forces from the limbs much lymph (Lassar, Cohnheim, Winternitz), as well as from the viscera; and, following this effect of direct pressure, there is a secondary increase in the flow from both sources, due to new lymph-production (Heidenhain, Starling). The territory of cell-supply opened for the first time, so to speak, by struggle, helps account for the greatly swollen cell-output.

(*c*) Following struggle, the cell-concentration and cell-output become for a time less than they would have been in the absence of muscular exertion.

This is demonstrated in the charts of Experiments IV, V and VI. The lessening in cell-output might be deemed merely such as was seen in the control animals of the first three experiments, were it not that the cell-concentration and the lymph-output (of which two the cell-output is the product) are both lower for a time than they would have been in the maintenance of quiet. One may suppose the glands to have been deprived of the majority of immediately available cells, and the slowed lymph-stream insufficient to wash with it all of those actually present.

These findings are, further on, dealt with in their clinical bearing.

Experiments in which the rapidity of the lymph's flow is varied without movement by the animal should provide increased light on the mechanism responsible for the results just described. The lymphagogue action of glucose was accordingly turned to this purpose.

The period of observation in these experiments was so short that it could not matter if the glucose acted to stimulate or retard cell-development. Other factors, though, demanded consideration. A possible effect of a lymph of high sugar-content to loosen elements from the glands, through changes in osmotic relations, could not be ruled out. Further, the lymph of struggle and of "glucosæmia" are not derived in similar proportions from the same regions.

There are three great areas of lymph-supply (Starling): The liver; the other abdominal viscera, in special the intestines, whence the lymph of the whole region may be designated "intestinal"; and the remaining portions of the body, the lymph from which may be termed "extremity-lymph." As has been said, the lymph of struggle comes from all of these sources, and in no small part from the limbs. The intravenous injection of glucose gives also increased lymph-flow from all the sources (Starling). The results of the procedures might, then, be directly compared, were the tissue forming lymph-cells equally distributed. But the liver of the dog possesses none of this tissue except that in the glands at its hilus (Ellenberger (27)), whereas the intestines and mesentery are

quite rich in it. The other body-parts possess, in proportion to their bulk, a very moderate quantity. Thus, of "mixed lymphs" from the thoracic duct, those derived most largely from the liver should be poorest in cells. So one must ask whether the lymphs of struggle and of "glucosæmia" are exactly similar in their derivation. To this only an approximate answer can be given. Starling has found that of the lymph obtained after the injection of glucose, much comes from the liver, less from the intestines, and relatively little from the other portions of the body. According to him increased blood-pressure and differences in permeability of the capillaries are responsible for the whole phenomenon. On the other hand the abrupt, initial increase in lymph-output induced by struggle is largely dependent on lymph previously present in the limbs, and now forced from them by the movements. Nothing analogous to this is caused by the glucose. The persistence of the large lymph-flow during struggle is traceable, however, to the same cause[5] as that following glucose injection, viz., increase in blood-pressure; and the resultant lymph is derived in much the same relative proportion from the three regions of production. Thus a rational basis is given to a comparison of the effects of glucose on cell-content with those observed in struggle after the initial increase in lymph-flow has subsided.

Experiment VII.—Mongrel; female; wt. 11.3 kilo; no food for 48 hours previous to experiment. The duct was ligated, and opened, 6 minutes before the beginning of lymph-collection. Lymph tinged with yellow, clear; no clotting in tubes or cannula. After three specimens had been got, 45 grammes of glucose in 72 c.c. of distilled water were injected slowly into the left subclavian vein. The animal remained absolutely quiet. The cell-counts were made in the order in which the specimens were taken, and 2½ to 3½ hours after their collection. (See Chart 7.)

At autopsy the animal proved to have been healthy; some round-worms were found in the intestines.

The results on cell-content are identical, as the chart shows, with those of struggle.

Experiment VIII.—Mongrel; female; wt. 16 kilo; no food for 48 hours previous to experiment. The duct was ligated during 10 minutes, and some stasis thus induced. On account of this, it was deemed safest to let the lymph

[5] This assertion might justly be objected to by those who oppose the theory of the mechanical formation of lymph. It stands or falls with that theory.

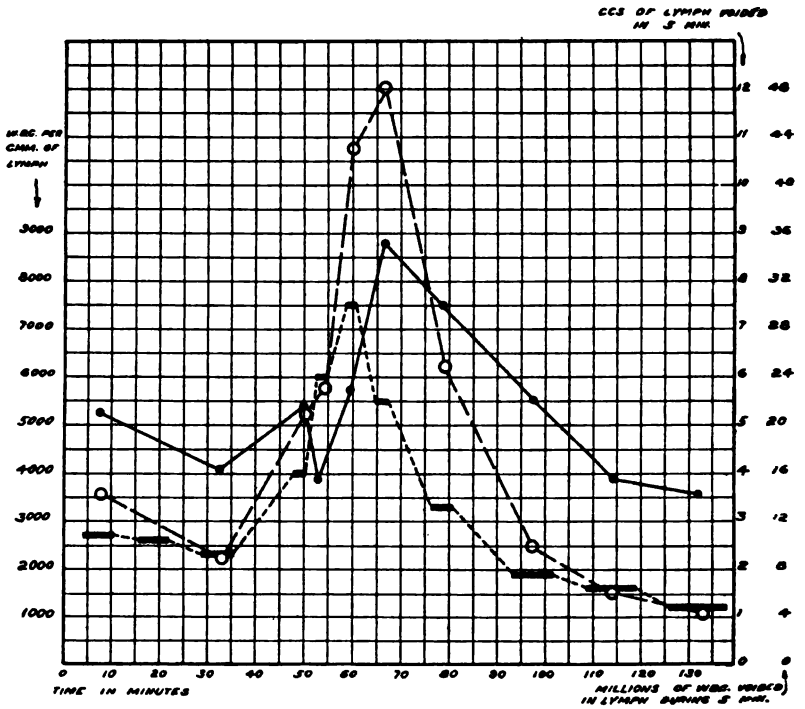

CHART 7.

escape for some minutes (12) before beginning its collection. Lymph clear; yellow-tinged; no clotting in tubes or cannula. After one specimen had been obtained, 52 grammes of glucose in 80 c.c. distilled water were slowly injected into the left subclavian vein. When the increase in lymph-flow due to this had subsided, the animal was made to struggle. Specimens were taken at frequent intervals. No chloroform was necessary, following its preliminary use to make anæsthesia complete. Cell-counts were made from the tubes in their order of collection and 2½ to 4 hours after that. (See Chart 8.)

At autopsy the animal was found to have been pregnant. There were ten embryos of an average length of 1 centimeter.

In this instance the effect of the glucose differed from that in Experiment VII. Here the cell-concentration rose, as result of the increased lymph-flow, whereas there it fell. In both cases the total cell-output became larger. In Experiment VII the curves were in all ways typical of those of struggle, whereas in Experiment VIII they were quite different, a fact which struggle in the same animal

helped to bring out. This struggle, coming after the glucose had operated, and causing an increase in the lymph but little superior to that from the glucose, was attended nevertheless by a cell-output vastly greater, and by the usual high cell-concentration.

These dissimilar results of glucose were puzzling. But the condition of the animal in Experiment VII had not been quite the

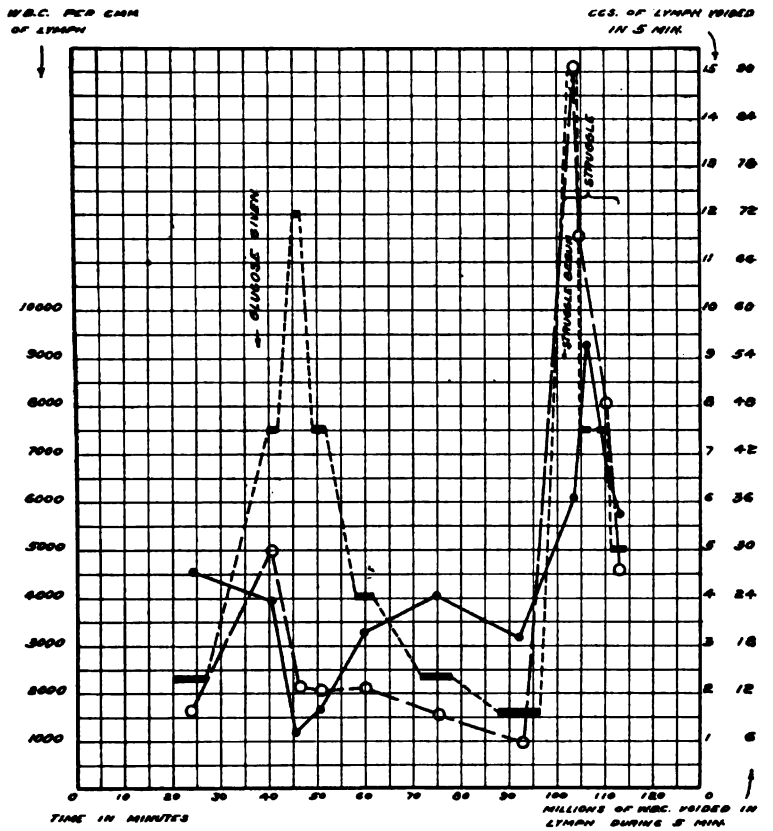

CHART 8.

same as that of the others. Owing to difficulty in the isolation of the thoracic duct, it had lain quiet under the anæsthetic 4 hours before the experiment proper began, instead of, like the others, only 1 to 2 hours. Perhaps, during this long period of preliminary quiet, the cells matured in the lymph-glands had in large part failed to be

carried away by the slow lymph-current, whence the marked appearance of them in the rush of fluid due to the glucose. On such reasoning it was determined to repeat the experiments, avoiding a long period of preliminary quiet, or flushing transiently the lymph-channels previous to the glucose injection by inducing restlessness in the animal.

Experiment IX.—Bull-dog; male; wt. 15 kilo; no food for 48 hours prior to experiment. The duct was ligated, and opened, 16 minutes before lymph-collection. The lymph was chyliform; no clotting in cannula or tubes. After one specimen had been obtained with the animal quiet, restlessness was brought

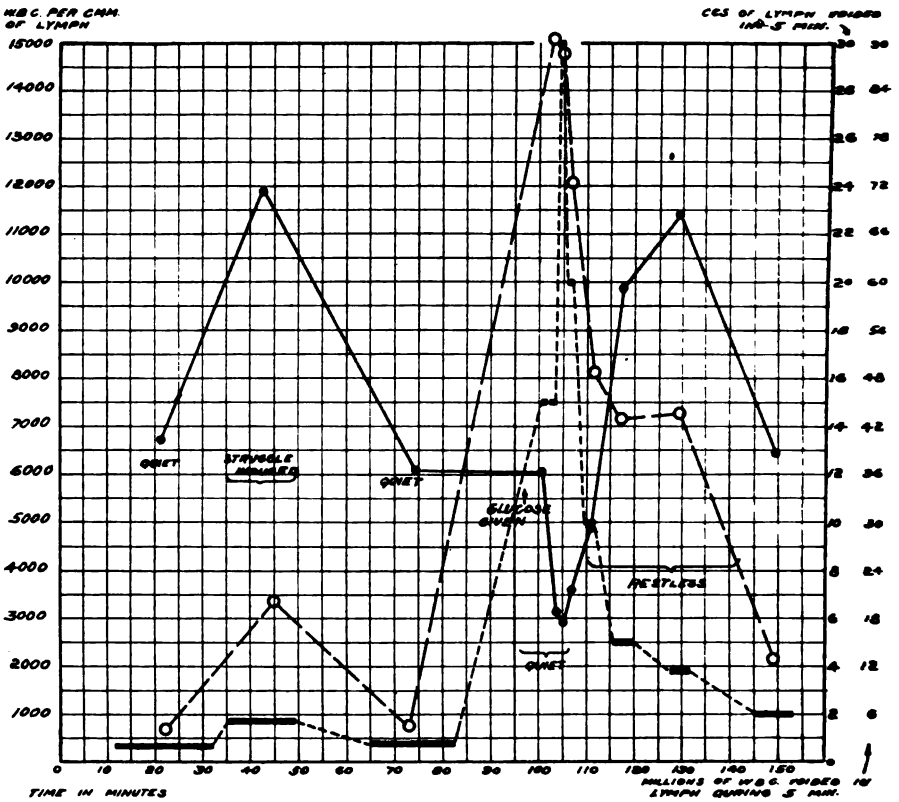

CHART 9.

about, in this case no real struggle, but a stiffening and straining, accompanied by labored respiration. The lymph became transiently more chyliform. A second specimen was now taken; on the return of quiet a third; and 50 minutes after restlessness had ceased 60 grammes glucose in 100 c.c. of distilled water were

injected slowly into the left external jugular vein. Before the lymphagogue action had disappeared the animal again became restless. Cell-counts were made in the order in which the specimens were taken, and 2½ to 3 hours thereafter.

The dog at autopsy proved to have been sound. Several large pieces of bone in the stomach accounted for the chyliform lymph. A tape-worm was found in the intestine.

From the chart it will be seen that struggle brought its characteristic effects on the lymph's cell-content, despite an extremely small increase in rapidity of flow. With the enormous lymph-output caused by the glucose, the concentration of the separate cubic millimeter of fluid was diminished, yet the cell-output as a whole became profoundly more.

Experiment X.—Mongrel; male; wt. 24 kilo; no food for 24 hours before experiment. The duct was opened 18 minutes previous to the beginning of lymph-collection. It had been ligated 8 minutes. After observations during quiet the animal was made to struggle during 2½ minutes, and 30 minutes later 100 grammes glucose in 100 c.c. of distilled water were injected into the left sub-subclavian vein. The lymph, which had been slightly opalescent, now became distinctly milky. There was no clotting in tubes or cannula. Except for the one struggle, the dog was quiet throughout. Counts of the tubes were made in the order of their collection and 1½ to 3 hours following that. (See Chart 10.)

At autopsy one tape-worm was found in the intestines.

No count was made of the lymph taken during struggle. The curves representing glucose action are similar to those of Experiments VIII and IX. In this instance such a rush of lymph was observed, and such a vast increase in total cell-output (despite lessened cell-concentration), as showed itself in no previous experiment. The curves representing these are much flattened.

The results justify the supposition that the high cell-concentration seen in Experiment VII is traceable to accumulation of cells during the long, preliminary quiet. Experiment VII shows that under certain conditions increased lymph-flow[6] may give results exactly similar to those of struggle.

To summarize the results with glucose:

(*a*) The increase in lymph-flow produced by the intravenous injection of a solution of glucose is accompanied by an alteration in the cell-concentration of the lymph. Usually the cell-concentration

[6] The change in osmotic relations caused by the glucose cannot be ruled out as a possible factor.

is decreased, but there is some evidence to show that, when the conditions have been such as to lead to the accumulation of cells in the lymph-system, it may be increased.

(*b*) With the increased output of lymph there goes an increase in total cell-output. This increase may be enormous. In Experiment X 5½ times as much lymph and 3½ times as many cells, were

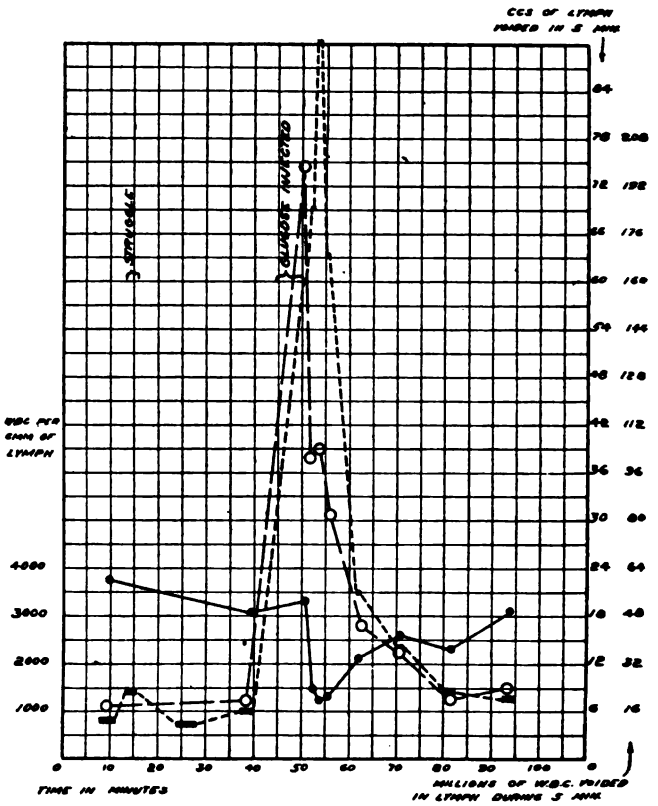

CHART 10.

voided in the half-hour immediately following the glucose administration as in the previous half-hour of quiet.

One may ask whether the results are not produced in the same way as those of struggle, and if they are not similar to them, except that the cell-concentration is rendered small by the distribution of the cells through a large amount of lymph. To put the question

directly, are the results of struggle those of simple increase in lymph-flow?

The evidence at hand is against this view. One may obtain a greatly increased cell-concentration during muscular activity in which the lymph-flow is quickened but little. Furthermore, increased lymph-flow by itself often diminishes the cell-concentration of the lymph, whereas struggle invariably heightens it. A direct comparison of the two procedures, such as Experiment VIII fortunately gave, demonstrates a difference in their results. In this experiment during the 11 minutes of muscular activity 19.25 cubic centimeters of lymph, with an average cell-content of 6877 cells per cubic millimeter, were voided; whereas, in the 11 minutes of greatest lymphagogue action, the nearly identical quantity of lymph voided (19.8 cubic centimeters) held only 2,267 cells per cubic millimeter. Differences in amount of lymph-flow alone could not be responsible for these variations of the cell-concentration in opposite directions and the differences in total cell-output.

It is true that, as already discussed, the lymphs obtained by the two methods may not be derived entirely from the same regions. The first "struggle lymph" is made up largely of that already present in the extremities, whereas that of the glucose is "liver-," "intestine-," and "extremity-lymph," in much the same relative proportions as that produced late in struggle.

It is questionable whether the cell-content of the "extremity-lymph" forced into circulation at the beginning of struggle could increase the cell-concentration of the "mixed lymph" of the thoracic duct, since it comes from a territory poor in lymphadenoid tissue, and has, indeed, been shown to hold normally fewer cells per cubic millimeter than does this "mixed lymph" (Pohl, Winternitz). But leaving this to one side, we are permitted a comparison of the effects of glucose and those that appear in struggle after the first rush of lymph has ceased. In the one instance the cell-concentration is diminished, in the other it is heightened.

One must conclude that another factor besides increase in lymph-flow per se helps during muscular activity to increase the cell-output. This is not hard to imagine. Direct pressure effects on the lymph-stream to set in motion those cells that had settled in the vessels,

and to scour the glands of mature elements, should certainly play a part. The work of Harvey (29) on the lymphocytosis caused by pilocarpine indicates that it is brought about by contraction of the smooth muscle in the capsules of the lymph-glands and spleen. Thus another possible action of struggle is suggested. Whatever the factor or factors may be, they are probably quite as influential during struggle to produce the large cell-output as is the accompanying increase in lymph-flow, which Ehrlich holds to be alone responsible for all heightened output of lymph-cells not dependent on their more abundant maturation. Nevertheless the theory that increase in lymph-flow gives increase in cell-output, is supported by the results of the lymphagogue action of glucose. The only objection to these as direct proofs of it is that an effect from the changes in osmotic relations brought about by a lymph of high glucose content cannot be ruled out.

It should be noted, in observing the charts as a whole, that the variations in cell-output are in keeping, quantitatively, with the amount of cell-output during quiet, the "cell-capital," so to speak. Succeeding to variations the cell-output tends to return close to its height previous to them. The fact that it usually becomes somewhat less than before them,—an indication of that gradual diminution in it observed in animals from which the lymph is gradually drained (Experiments I, II and III), or, in some cases (Experiments V and VI) of partial exhaustion of the supply of mature cells, —does not affect the principle. We may say that the cell-output seems "set" to maintain a stable rate during some hours at least. Healthy, adult dogs, kept, so far as possible, under the same conditions, differ widely in this rate of cell-output: in Dog Gl more than nine times as many lymph-cells per kilo of body-weight are furnished to the blood through the thoracic duct as in Dog Fl. Does this mean a difference in amount of cell-production by the tissues? The appended table helps answer this question.

From this table it is clear that, while the cell-output per kilo of body-weight does not depend on size of the individual, or on differences in length of fast,[7] it has perhaps a relation to rate of lymph-

[7] Firleiewitsch (30) has found the lymph-glands of well-fed rats to be more numerous and larger than those of starved ones; but he assigns this to a larger size of the cells making up the tissue, not to a greater number of them.

Dog.	Length of Fast.	Weight in Kilos.	Average No. of w. b. c. per cmm. Lymph.	Average Flow of Lymph in 5 Minutes.	Total w. b. c. in this Amount of Lymph.	Total w. b. c. in Amount of Lymph Furnished per K. of Body-weight in 5 Minutes.	Flow of Lymph per K. of Body-weight, in 5 Minutes.
Ll, bull, male	48	15	6,700	0.7	4,690,000	312,666	0.05
Hl, terrier, male	24	11	4,960	0.75	3,720,000	338,181	0.07
Pl, collie, male	48	13	2,900	1.5	4,350,000	334,615	0.11
Al, bull, female	24	15	3,600	2.0	7,200,000	480,000	0.13
Kl, collie, male	48	16	4,510	2.3	10,373,000	648,313	0.14
Gl, bull, male	24	18	11,160	3.3	36,828,000	2,046,000	0.18
El, setter, male	24	20	8,400	3.75	31,500,000	1,575,000	0.19
Ml, setter, male	24	24	3,780	4.6	17,388,000	724,500	0.19
Ol, collie, male	48	23	6,760	4.6	31,096,000	1,352,000	0.20
Cl, mongrel, male	2	10	1,500	2.0	3,000,000	300,000	0.20
Nl, terrier, female	48	8	4,180	1.7	7,106,000	888,250	0.21
Jl, mongrel, male	48	11.3	4,640	2.5	11,600,000	1,026,540	0.22
Fl, mongrel, male	24	19	990	4.3	4,257,000	224,053	0.23
Il, spaniel, male	24	11	3,000	3.8	11,400,000	1,036,363	0.34

flow per kilo of body-weight, being, on the whole, least in those individuals in which this flow of lymph is least. It may be supposed, in the light of what has gone before, that the lymph-flow, as an agent of transportation, is here responsible for the differences in cell-output, rather than by stimulation of the cell-forming tissues; in special since, so far as we know, this rate of cell-output is constant only from hour to hour, and may not be so from day to day. Some of the wide differences may be due to the presence of an accessory thoracic duct, which is not infrequent in the dog (Biedl and v. Decastello), or to other channels conveying a share of the elements that would, normally, course through the thoracic duct. In association with the idea of actual variation in the productive activity of the cell-forming tissues, may be cited the work on ruminants of Forgeot, already quoted. He found the cell-output of young individuals to be markedly greater than that of adults. Differences in age of the animals may be at the root of some of the differences in cell-output. But the mechanical factors, just cited, will explain the larger differences, and make needless a further entrance into the dark subject of activity in the cell-forming tissues.

The cell-concentration of the lymph of the dog, as determined under the conditions of operation outlined at the beginning of this paper, has a worth in its relation to the cell-content of the blood.

Delamere, Winternitz, Goodall and Paton, Dastre, Henri and Stodel, Beidl and v. Decastello, and Ranvier give figures ranging from 1,372 to 22,729 white cells per cubic millimeter. The results in the table, as might have been expected from the technic employed, do not exhibit such wide variation. From them one may judge this "normal" cell-concentration of the "mixed lymph" of the dog to lie between 990 and 11,160 cells per cubic millimeter, with an average of 4,000 cells.

The most important outcome of this work is the discovery that the system forming the lymphocyte possesses large reserve power to increase transiently its output of cells. During muscular activity this may, for the space of a half-hour, be nearly four times what it is in quiet, as has been shown. A clinical application of the finding is not far to seek.

For this application it is necessary to know what white cells the lymph furnishes the blood. These are in the dog much the same as in man, just as the leukocytes of the dog's blood resemble in general those of man both in morphology and in relative proportion of number (Dawson (31), Tallquist and Willebrand (32), Busch and Van Bergen (33)). The bulk of cells furnished through the thoracic duct of the dog is one of lymphocytes, large and small, but a few large mononuclears, a varying, small percentage of eosinophiles, and an occasional polymorphonuclear neutrophile are also present (Delamere, Biedl and v. Decastello). The lymphocytes alone exist in sufficient quantity to be important to the blood.

Since, as has been emphasized, the thoracic duct furnishes a large, if not the greater part, of the blood-lymphocytes, an increase in the lymph's cell-concentration should produce, other factors being equal, an absolute lymphocytosis in the blood. When the amount of lymph is at the same time increased, thus multiplying the cell-output, the effect should be more profound. Thus one would expect struggle to produce, clinically, an absolute lymphocytosis.[8]

[8] But it must be assumed that the change in cell-output is not accompanied by change in cell-formula. I have made repeated counts of the lymph before and during struggle, and have found no such change.

The clinical records of blood-counts following muscular exertion indeed show the presence of an absolute lymphocytosis. Schultz (34), and later Winternitz, observed a leukocytosis following exertion, but they did not note its kind. Burrows (35) found in the normal individual, after short exercise, a distinct increase in number of all the white cells, but especially of the lymphocytes. Capps (36), previously, had studied the blood during the convulsions and apoplectic attacks of general paresis, and, here too, had observed leukocytosis, most marked in the large mononuclear elements, but affecting the lymphocytes. Burrows went further in making plain the fact that the leukocytosis associated with true convulsions in the course of paresis is invariably of the inflammatory type, the polymorphonuclear neutrophiles giving the increase. He concluded, from his findings made during muscular exertion, that in convulsions two leukocytoses are really involved, a transient "physiological," wherein occurs an increase of all the elements, most marked, as his records show, in the lymphocytes, and a more pronounced and enduring "pathological" one.

Violent and long-continued physical exertion will itself produce a profound leukocytosis. Thus Larrabee (37) observed it as an effect of a 25-mile foot-race; and he decided that, here too, a "pathological" is superimposed upon a "physiological" leukocytosis, "an increase in cells all along the line," as he puts it. Only this "physiological" leukocytosis is of interest here. Larrabee, Tileston and Emerson (38), studying the blood after a similar race, confirmed these results. Further, the lymphocytes at the end of such exertion form a very small percentage of the total, having retreated to their absolute number during quiet, or even below it. We do not know how far destruction of the lymphocytes in circulation effects this and how far it is brought about by a lessening in the supply of them. The experiments reported in this paper show that a diminution in the cell-output by way of the lymph follows prolonged struggle.

Coming to more debatable ground, the well-defined lymphocytosis that accompanies whooping-cough may be referred to. This cannot be primarily due to the mechanical effects of struggle, since it appears early in the disease; yet the fact that it is at its greatest during the period of violent coughing (Meunier (39)), is suggestive. Un-

fortunately there are no published figures dealing with the leukocyte count immediately before and after a coughing-fit.

The experiments with a lymphagogue throw a little light on the vexed subject of digestive leukocytosis, particularly on the reason that it is vexed. The frequency of this leukocytosis, and the part played in it by the mononuclear element, varies with nearly every investigator who has applied himself to the problem. The discordance in results is, to a certain extent, explicable in terms of the conditions dealt with here. Quiet previous to a meal would predispose, on increase of the lymph flow during digestion, to a cell-output such as that in Experiment VII, which would probably swell quite markedly the number of lymphocytes in the blood. Any exertion after the meal would tend to make this output greater. Exercise previous to a meal, by flushing out the reserve of mature cells, would act to prevent an increase in cell-output during digestion, and this result would be made the surer by slow lymph-flow, were but little fluid ingested with the meal. Under such circumstances the blood-content in lymphocytes would not increase.

I am aware that, considering the many factors which must enter into the determination of the blood's content in lymphocytes, this discussion is one-sided. Yet, whether the hypotheses presented above be sound or not, the work on which they are based indicates one direction in which it may be possible to simplify some of the problems connected with the leukocyte.

CONCLUSIONS.

1. The lymph of the thoracic duct furnishes to the blood a larger proportion than is usually supposed of the lymphocytes in circulation. Gross variations in its output of such cells must affect very considerably the blood picture.

2. The quantity of lymphocytes supplied through the thoracic duct of the healthy dog remains practically constant from hour to hour, if the physiological conditions are not notably changed. Transient change in physiological conditions may alter the output of cells, but with the disappearance of this change the output tends to resume its previous rate. These facts indicate that the tissues producing lymphocytes are "set" at a rate of activity definite in the individual.

3. Muscular activity (struggle) produces a prompt increase in the output of lymphocytes through the thoracic duct.

(*a*) This is assured by the presence of an increased number of cells per cubic millimeter of lymph, combined with an increase in the amount of lymph voided.

(*b*) The lymphocyte-output may be tripled or quadrupled during a long-continued struggle.

(*c*) Following prolonged struggle the output of lymphocytes is for a short time less than previous to the exertion.

4. The increased lymph-flow caused by a lymphagogue of the second class (glucose) brings with it increased output of lymphocytes through the thoracic duct.

(*a*) The individual cubic millimeters of lymph are often poor in cells, during the rapid lymph-flow, yet the total number of elements transported is large.

(*b*) The results with glucose support the theory of Ehrlich, that a rapidly appearing lymphocytosis may be produced through the flushing effect of increased lymph-flow.

5. A comparison of the effects of struggle with those of glucose demonstrates that in the former some factor besides increase in lymph-flow per se (Ehrlich) works to cause the large output of lymphocytes. The nature of this factor has not yet been determined.

6. The variations caused by muscular exertion and by increased lymph-flow in the number of lymphocytes coursing through the thoracic duct are so pronounced as to suggest that the total number of lymphocytes in circulation must be considerably influenced by them. Clinical findings by other observers indicate that this is true; and the clinical findings themselves become much simpler of interpretation.

7. The results in general prove the existence, reserved from circulation, of a large fund of lymphocytes, which is quickly yielded to the blood under certain physiological conditions.

I wish to thank Dr. Warthin for an interest in the work that has been most helpful.

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CONCERNING THE RELATION OF THE COAGULATION TIME OF THE BLOOD TO THROMBOSIS IN PHLEBITIS.[1]

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The etiological factors or conditions concerned in the production of thrombosis may be briefly summarized as:

1. Central or peripheral slowing of the blood stream.
2. Lesions in the walls of the blood vessels.
3. Alterations in the blood itself, such as tend to favor coagulation.

In actual cases, it appears almost without exception that two or more of these factors are associated in the production of thrombosis.

Although the etiological agents mentioned above are generally accepted as correctly explaining thrombosis, it must be recognized that experimentally as well as clinically very uncertain results follow when we so attempt to explain concrete instances, notably such as occur in clinical phlebitis. Until more certain data are secured in regard to this process, but little can be expected in the way of successful prophylactic treatment or in the certain determination of those instances in which this lesion is to be feared.

The object of this brief study has been an attempt to show what part artificially increased and decreased coagulability of the blood plays in the production of thrombosis, or, expressed in other words, whether in conditions productive of phlebitis, thrombosis is more likely to occur when the coagulation time of the blood has been lowered artificially or less apt to take place when analogous means have been employed to prolong the coagulation time of the blood.

Our experiments have been conducted on a series of young and

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healthy rabbits, one third of which had their coagulation time reduced by daily administration of 2 grams of calcium lactate; an equal number of animals in which the coagulation time had been artificially lengthened by daily dosage of 2 grams of citric acid; the remaining third was used as control animals. The drugs were introduced into the stomach by tube feeding and it was found possible, with this dosage, to reduce the coagulation time of the blood in the calcium lactate animals one half and to lengthen it in the citric acid animals about one third. As corroborative of work already well authenticated, we found that the maximum effect of these drugs takes place about two hours after their administration and probably passes entirely away within twelve hours, especially in the case of the rapidly excreted calcium salts. In our animals the medication was continued in daily doses throughout the experiment, since it was found that effects were quite as likely to occur several days after the initial injury as immediately on its infliction, thus also more closely approximating the conditions occurring in man.

When autopsies were to be performed the animals were chloroformed and while the heart action was still vigorous a carotid artery was opened and the animal suspended so that the blood was very generally emptied from all the vessels of the body, and post-mortem clot or fibrin could not become confused with true thrombi. We believe this to be an important technical step.

Our experiments may be grouped in two series:

Series I comprises local injuries produced in and around the ear veins. Nine experiments of this character were performed.

Series II includes attempts to produce vascular lesions predisposing to phlebitis and thrombosis by intravenous injections of various irritants. Five sets of experiments were made.

SERIES I. *Experiment a.* A 3 centimeter segment of the distended marginal ear vein was isolated by compression between two artery clamps and the intervening vein distended with blood was compressed and lacerated with toothed forceps for five minutes when minute hæmorrhagic extravasations along the course of the trunk were demonstrable. The isolating clamps were then removed and the circulation allowed to become reestablished. The resulting peri-venous inflammation was slight and no thrombosis followed either in the calcium, citric acid or control animal.

Experiment b.—Clamping the marginal vein with ordinary paper clips, cutting off at the same time the anastomosing circulation for thirty minutes, was followed by immediate reestablishment of the normal blood flow in all animals without subsequent results.

Experiment c.—A 2 centimeter segment of the marginal vein was clamped and isolated with paper clips for twelve hours, the anastomosing circulation being meanwhile prevented. Immediately on the removal of the clips the circulation was reestablished in all animals. The absence of thrombosis in these experiments was similar to the results of the experiments reported by v. Baumgarten (1) and his student, Rizor (2), who was able to compress the vein for an even greater length of time without resulting thrombosis. After three days considerable inflammatory reaction developed about the site of some of the clamps, being most marked in the calcium lactate animal, where finally a small segment of the vein became thrombosed. The inflammatory reaction was not so marked in the control animal and there was still less reaction in the citric acid rabbit, thrombosis being absent in both.

From these experiments one may conclude that mere stagnation of the venous blood produced no marked tendency toward thrombosis in the ear veins but that inflammatory lesions, with consequent phlebitis are more extensive in the case of the calcium animal while thrombosis occurred only at the immediate point of injury of the vessel walls.

Experiment d.—A quantity of 24-hour growth of virulent pneumococci in bouillon was injected about the ear vein, the injection being continued to such a point as to cause compression anæmia of the desired segment of the vein. The circulation was shortly reestablished and the amount of subsequent inflammatory reaction was slight. No thrombosis occurred in any of the animals. Our results in this experiment are therefore quite unlike those of Talke (3), where, however, the coagulation of the blood was not altered, for Talke claims to have regularly produced thrombosis in this way.

Experiment e.—Five minims of 5 per cent. silver nitrate solution were injected into the peri-venous connective tissue of the ear. Immediate permanent thrombosis of the adjacent vessels followed. No difference existed in extent or degree between the three animals. This experiment was repeated using 1 per cent. solution of silver nitrate. Slight peri-vascular inflammation without thrombosis resulted and was of about equal severity in all the animals.

Experiment f.—Three drops of pure turpentine were injected between the branches of the median ear vein. This was followed within twelve hours by œdema and an active inflammatory exudation with thrombosis of the involved vein in all three animals. The thrombosis was notably more extensive and resolution most delayed in the animal which had received the calcium lactate, less so in the control and least of all in the rabbit which had been poisoned with citric acid.

The same relations, as regards severity and occurrence of lesions, followed when 50 per cent. turpentine in an inert oil was

employed in the experiment except that the resulting inflammation and subsequent thrombosis was longer delayed.

SERIES II.—Finely comminuted sterile pumice was injected into the marginal ear vein in an attempt to see if the resulting thrombosis, following probable embolism of the terminal arterioles, would be more extensive in the animal that had received the calcium salt and less in the one that had received citric acid. All three animals recovered perfectly from the operation and later autopsies showed no lesions whatsoever which could be attributed to this injection.

Experiment b.—Fifteen minims of sterile cod liver oil were injected into the marginal ear veins of the three animals. None of them showed symptoms and later post mortems showed no lesions attributable to the experiment.

Experiment c.—Ten minims of a pure 24-hour culture of virulent typhoid bacilli were introduced through the marginal vein. No symptoms of illness followed and these animals were subsequently utilized in experiment *d.* These results are exactly contrary to those of Jakowski (4) who in similar experiments on guinea-pigs and rabbits obtained almost constant thrombosis, without the associated employment of calcium or citric acid.

Experiment d.—Fifteen minims of a suspension of a 36-hour broth culture of virulent typhoid bacilli in an equal bulk of sterile cod liver oil were introduced through the marginal ear vein. This was done in the expectation that embolism caused by the oil would be likely to afford sites for the growth of the bacilli thus leading to thrombosis as described in the experiments of Jakowski (4). All the animals became seriously sick and died, on postmortem examination multiple serous petechiæ, general parenchymatous degeneration, lymphadenitis and frequent infarctions were found, but no general or isolated thrombosis. The lesions were notably most extensive in the calcium lactate animal and least extensive in the citric acid, but when the experiment was repeated under similar conditions, exactly opposite results followed, which leads us to believe that chance was really the controlling factor in determining these changes.

CONCLUSION.

Positive results have been obtained in but a single set of experiments, namely those in which turpentine was employed.

In so far as the results of this preliminary study go, one is led to the conclusion that thrombosis is most readily induced when active inflammatory lesions exist in the blood vessels, associated, probably in most instances, with secondary degenerative changes. Purely mechanical lesions are much less apt to be productive of conditions favorable to thrombosis as a sequence of phlebitis.

Marked artificial increase or decrease in the coagulation time of the blood by the use of calcium lactate or citric acid, does not render animals abnormally prone to thrombosis incited by changes other than inflammatory.

When true phlebitis exists, thrombosis is apt to be more extensive and less readily resolved, when the coagulation point of the blood has been shortened by the use of calcium lactate, and it is less extensive and more quickly absorbed when the coagulation time has been increased by the administration of citric acid.

Experiments as yet incomplete appear to suggest that the increasing in rapidity or slowing of the general circulatory stream has but little bearing on the production of thrombosis in phlebitis, much less, indeed, than clinical and anatomical observations have generally led us to think. We have also been led to suspect that the presence or absence of anastomoses of abundant degree is largely concerned as a factor in determining the location and extent of thrombosis in phlebitis.

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CALCIFICATION OF THE ARTERIAL SYSTEM IN A CAT WITH TRANSPLANTED KIDNEYS.[1]

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In a previous paper,[2] the history of a cat,[3] whose kidneys were extirpated and replaced by the kidneys from another cat, and whose arteries underwent afterwards extensive calcification was incompletely reported. It was the first observation showing that a very slight morphological change of the kidney might be followed by intense and rapid arterial degeneration. On account of the importance of this fact it seemed advisable to summarize the clinical history of the animal and to describe fully the gross and microscopical findings of the autopsy.

The animal operated on was a young adult female cat in excellent health which had lived in the laboratory for several months. Her kidneys were resected, the abdominal aorta was dissected and found normal, and both kidneys from a middle aged female cat, which was also in good health and whose arteries were normal, were grafted into her abdominal cavity. The animal recovered quickly from the operation and her life went on just as before. Fifteen days after the operation, both kidneys were movable and normal in size. The animal urinated and lived as a normal cat. Seventeen days after the operation, both kidneys were found very much enlarged and fixed to the lumbar wall, and the urine contained a great deal of albumin. An exploratory laparotomy, performed on the eighteenth day, showed both kidneys very much increased in size. Their consistency was softer than normal. The lumbar peritoneum was incised on the middle line, and dissected. The arterial and venous circulations appeared to be normal. The connective tissue of the hilus was œdematous, and clear fluid flowed

[1] Received for publication, January 7, 1908.

[2] Alexis Carrel, *Jour. of Exper. Med.*, 1908, x, 98.

[3] Experiment 14.

from it. The wall of the ureter was œdematous also. The color of both kidneys was rosy and normal; there was no congestion. The peritoneum covering the anterior face of the right kidney was dissected and retracted and the capsule incised; clear fluid and red blood flowed from the incision. Then the renal tissue was incised and found œdematous, but not congested. An abundant hemorrhage of red blood followed, which was controlled by suture of the capsule with very fine silk. No suture of the lumbar peritoneum was made. The abdominal wound was closed as usual. After the operation, the quantity of albumin decreased rapidly. The size of the kidneys diminished progressively and was almost normal fifteen days afterwards. Nevertheless the animal became emaciated, and, without having presented any definite symptoms, died on the thirty sixth day after the transplantation.

The autopsy was performed one hour and a quarter after death.

Macroscopical examination.—On the abdominal wall, two transverse scars are observed. The lower one is linear and hardly discernable; it is the scar of the first laparotomy. The upper one, a little wider and very apparent, is the scar of the second laparotomy. To palpation, these scars present extremely different characteristics. The scar of the first laparotomy is a normal, narrow and elastic scar, while the scar of the second laparotomy is very wide, irregular, and its consistency is extremely hard, as if a rib had developed in the abdominal wall.

After incision of the wall, it is found that the scar of the first laparotomy is entirely normal, without any apparent infiltration of lime salts. On the contrary, the scar of the second laparotomy is so hard that it is difficult to cut it with the scissors. The wall is infiltrated with masses of lime salts, white in color and almost as compact in structure as a stone of the urinary bladder. Nevertheless, at the level of this scar, the peritoneum appears to be normal. It presents a white color, which is due merely to the deposit of lime salts in the subperitoneal connective tissue.

The abdominal cavity was opened by a large transversal incision. There are a few adhesions of the great omentum to the scar of the second laparotomy and no adhesions of the intestine. There is no fluid in the abdominal cavity and the parietal peritoneum is normal everywhere.

The small intestine was eviscerated. Its connections are normal. However, a loop of the first part of the jejunum is adherent to the scar of the lumbar peritoneal incision made during the second operation, and is sharply kinked at the level of the lower part of this incision. Nevertheless, there is no obstruction of the lumen of the intestine. The large intestine is normal. The omentum and mesentery are normal. The superior mesenteric artery is larger than normal; its consistency is very hard, and it can be broken by pressure as though it were a glass tube. It seems also that the consistency and size of the smaller mesenteric branches are markedly increased.

The stomach is apparently normal, but its arteries are dilated and very hard. The liver, the spleen and the pancreas are normal.

The kidneys are in their normal position and covered with normal peritoneum. The cat being emaciated, there is little adipose tissue around the kidneys, and the peritoneum is very transparent. The color of the organs is normal, and also their consistency. Their size is slightly enlarged, but within normal limits. They are movable on the lumbar wall, as normal kidneys are. The loop of jejunum which was adherent to the incision of the second lumbar peritoneal incision having been detached, it is found that the scar of this incision is markedly infiltrated with lime salts. By examining more carefully the peritoneum covering the right kidney, a white longitudinal spot is seen about two centimeters external to the hilus. This spot is hard in consistency and composed of a deposit of lime salts in the subperitoneal connective tissue, between the peritoneum which is sound and the capsula of the kidney which also is normal. This deposit of lime salts corresponds approximately to the place where the vertical subperitoneal exploratory incision of the right kidney was made. The healing of this incision has been so perfect that very little evidence of it can be found by a most careful examination in the capsule or in the renal substance. Its location is marked only by the infiltration of lime salts into the subperitoneal tissue, which has followed probably a very small hemorrhage from the line of suture of the capsule. The renal veins and arteries are normal in size, direction and consistency. The connective tissue surrounding them is normal also. The transplanted segments of aorta and vena cava are normal, and the anastomoses are excellent. But, the line of suture of the arterial anastomoses is as hard as a ring of wire, and there is a sharp difference at this point between the transplanted aorta which is elastic and soft, and the host's aorta which is as hard as glass.

The ureters are normal. A few centimeters below the kidneys, they become adherent to each other and enter the peritoneal cavity through the lower part of the lumbar peritoneal incision. After having passed along the right side of the rectum and above the uterus, they reach the bladder. They are in excellent condition and their small vessels appear to be normal. The transplanted flap of bladder is perfectly united to the bladder which is normal in appearance and in size. Its anterior face is longitudinally incised. About five cubic centimeters of yellow clear urine, containing albumin are found. The openings of the ureters are distinctly seen and are normal. The transplanted mucous membrane is normal and the scar between it and the mucous membrane of the host is almost undiscernable.

Then the kidneys were dissected and directly examined. The capsule is normal in appearance. At the level of the anterior face of the left kidney, there is a narrow brownish line which is probably the scar of the exploratory incision. The adhesion of the capsule to the kidneys is normal, and not increased at the level of the scar. There is no dilatation of the stellate veins. Both kidneys were opened. There is no dilatation of the pelvis. The relative dimensions of the cortex and medulla are normal. The medulla is normal. The cortex is normal but a little pale.

The suprarenal glands are normal.

In opening the thoracic cavity it is found that the cartilaginous part of the ribs is very hard and friable and intensely calcified. The internal mammary arteries are also calcified. The pleura, the trachea and the lungs are apparently normal. On a macroscopic section, the pulmonary tissue seems normal, but, on palpation its consistency is found to be greatly modified by the presence of a great many very small and hard foreign bodies. These were proved to be the calcified ends of the small bronchioles. The larger bronchi are normal, and their rings do not present any calcification. However, thick calcific nodules are found from distance to distance on the external layer of the larger bronchioles.

The heart is very small, much smaller than the heart of a normal cat of similar size. There is no apparent calcification of the coronary arteries. The valves are normal. Nevertheless, as the aorta is very much dilated, there was probably a marked degree of aortic insufficiency. The arch of the aorta, the brachio-cephalic and carotid arteries, and the descending aorta, have the consistency of glass tubing and are exceedingly friable. The arch of the aorta was incised longitudinally. It is very hard to cut with the scissors, especially near the heart just above the sigmoid valves. There is at this level a very large accumulation of lime salt. The dimensions of the vessel are very much increased. The internal circumference of the aorta near the heart is 26 millimeters, the same dimension in a normal cat being about 18 millimeters. The internal surface is of a yellowish-white color, with a reticular appearance, the elevated parts being more yellow and harder, while the depressed parts are whiter and a little softer. It corresponds to the differences in the intensity of the lime infiltration. But the wall, over the entire circumference of the vessel, is infiltrated and rigid. It is not a lesion localized in a few patches, but a diffuse one generalized over the whole vessel. However, near the mouth of the brachio-cephalic arteries there are thicker rings of lime infiltration. On the descending aorta, the infiltration is a little less marked and more regular.

The wall of the aorta is thinner than normal in several places. On the upper part of the arch, which is very much dilated, and, just in front of the mouth of the left brachio-cephalic artery, there is a part so thin that it would have probably broken under the aortic pressure had the animal lived a little longer.

The lesions of the pulmonary artery are very different. The dilatation is not marked and the wall has an almost normal consistency. But there are intense focalized calcific lesions. Just above the sigmoid valves, on the convex part of the vessel, there is a long calcified patch of yellowish color, seven millimeters in length, and raised above the intima to a height of more than one millimeter. Other patches are observed near the bifurcation of the pulmonary artery and in its branches. Between the patches the wall seems normal. The smaller branches of the pulmonary arteries are found enlarged and infiltrated with small white patches.

Diffuse calcification and thick circular rings are found on the wall of the carotid arteries. The subclavian and humeral arteries assume the same appearance. Even the small muscular branches of the humeral arteries are calcified.

The abdominal aorta presents diffuse lesions almost similar to those of the carotid and subclavian arteries, that is, diffuse calcification with thicker rings from distance to distance. These lesions stop sharply at the point of the anastomoses, and the transplanted aortic segment is soft and normal.

The calibre of the abdominal aorta is very much increased. The cœliac artery is calcified and dilated. The upper mesenteric artery is also completely calcified, and its lumen is as wide as this of a normal aorta.

The iliac and femoral arteries are affected with similar lesions.

Microscopical Examination.—The specimens were fixed in Zenker's fluid and in formalin and stained in hæmatoxylin and eosin. The arteries were cut without having been decalcified. Some of the arterial sections were also stained with Weigert elastic tissue stain. Dr. Simon Flexner had the kindness to look over the sections for me.

The Kidneys.—Beneath the renal capsule, in the superficial part of the cortex, there is a marked œdema which extends about one half millimeter downwards and then disappears. It is not regularly diffused but is more marked in some places than in others. There is also a focalized increase in connective tissue immediately beneath the capsule, chiefly about greatly dilated blood vessels, with heavy fibrous walls. The latter are dilated superficial veins. Surrounding them, there is a rich infiltration of small round cells. In one of these small veins, an organized thrombus projects into the lumen of, but does not occlude the vessel.

The secretory tubules are on the whole remarkably well preserved and there is no increase in connective tissue around them. On the other hand, groups of collecting tubules, high in the cortex, occasionally show an increase in the surrounding connective tissue, dilatation and either casts or cast material, which are composed in part of disintegrated blood corpuscles. Following the collecting tubules into the medulla, one finds focalized infiltrations around them of small round cells.

In the glomeruli, Bowman's capsule is not increased in thickness. For the most part, the capillary loops fill the capsule. The endothelium stains sharply and the capillaries contain blood corpuscles and no excess of leucocytes. The capillary walls are not thickened. There is no fluid in the capsular space.

No calcification is detected in any of the renal structures.

The liver, the spleen, and the intestines are normal. There is no change in their small vessels.

There is no lesion of the lungs. But the cartilaginous rings and plates of the middle sized and small bronchioles are intensely calcified. There is no calcification of the rings of the large bronchi and of the trachea. A section of the upper part of the cervical medulla is found normal and there is no lesion of its small vessels.

The suprarenal glands are entirely normal.

The Arteries.—The segment of aorta which was examined belonged to the middle part of the descending thoracic aorta. The intima appears normal. The muscular coat shows a very great reduction in the number of the nuclei ordinarily present. This reduction begins to be visible in the layer beneath the endothelium and becomes progressively more marked as we proceed outwards toward the adventitia. As the nuclei disappear, the muscular cells become more and more confluent and converted into a highly refractive material. As the adventitia is approached, lime salts begin to be deposited. The external musculo-elastic layers of the media, and the adventitia are extensively infiltrated with lime salts. There is a less degree of calcification, consisting of the deposit of fine

lime granules, in the middle part and more internal part of the media. This latter is not uniform and does not entirely enclose the vessel. Where the lime deposit is greatest, there is an abundant infiltration of small cells, some of which show polymorphic nuclei. The cells are in a poor state of preservation, and these focalized areas correspond more or less closely to the so-called atheromatous abscesses. A section stained for elastic tissue shows that the coarser elastic fibers remain but are especially prone to rupture in the course of section. In the location described as resembling atheromatous abscess, the elastic tissue is deficient or very much broken up. The smaller blood vessels about the aortà do not show any calcification.

The carotid artery shows a condition somewhat different to that of the aorta. The entire muscular coat of the artery has lost its nuclei. Not one remains. It is converted into an homogeneous structureless membrane richly infiltrated with lime salts. The adventitia is relatively free of calcification. The elastic tissue stain shows that the elastic framework remains but the fibrils have coalesced together into stands of greater or less thickness, showing in transverse sections a parallel arrangement. There is no fine network present.

No section has been made at the level of the most marked lesions which took place on the arch of the aorta, and on the pulmonary artery.

No attempt will be made to explain the genesis of these degenerative arterial changes. The problem contains too many unknown quantities, and hypothesis, however ingenious, would have very little objective value. Nevertheless a few details of this observation must be emphasized, especially as regards the time of occurrence of the calcification, its localization, and its relation to the renal lesions.

During the first fifteen days following the double transplantation the animal was in normal condition and no calcification took place as was shown by the anatomic conditions at the second laparotomy and by the normal structure of the scar of the first laparotomy. Then, the kidneys became suddenly enlarged, and the lesion noted at the laparotomy performed on the eighteenth day was œdema of all the transplanted tissues, for which no mechanical cause was discovered.

The calcification took place during the eighteen days which elapsed between the second laparotomy and the death of the animal.

The calcification was localized to the arteries, costal and bronchial cartilages, and to the points where the tissues had been disturbed by incision, or even merely by infiltration of a little blood. It was a remarkable fact that no calcification was found in any of the transplanted tissues. The transplanted segment of aorta,

and the renal arteries were normal. The subperitoneal connective tissue of the host and the connective tissue of the hilus of the right kidney were dissected during the second operation. However the connective tissue of the hilus was found normal at the autopsy, while the subperitoneal tissue was infiltrated with lime salts.

The exploratory incision of the right kidney was perfectly cicatrized, and there was no calcification at all of the renal tissue or the capsule. But at the level of the incision in the subperitoneal connective tissue there was a patch of lime salts. The blood, which flowed from the edges of the renal structures, had produced a deposit of lime salts in the tissue of the host, while it was harmless for the transplanted tissue.

It must be noticed also that the pathological changes undergone by the renal tissue were slight. However, the kidneys are evidently responsible for the occurrence of the arterial degeneration, directly or indirectly. Therefore, this observation seems to show that some unknown condition of the kidneys corresponding merely to unimportant morphological changes, can produce rapid, grave and extensive arterial lesions associated with deposits of lime salts in them and in other locations.

THE SO-CALLED RHYTHMS OF GROWTH-ENERGY IN MOUSE CANCER.*

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PLATE XX.

The cause of carcinoma—the subtlest of human diseases to-day—is essentially a biological problem, the attempted solution of which has drawn out many wild guesses, not a few misapplications of biological principles, and some good working hypotheses. Stated in its simplest form, the problem is to find out what causes the continued division of the epithelial cells of the tumor. Albrecht,[1] writing of tumors, quotes Berkeley to the effect "that we have raised a dust and then complain that we cannot see," and uses this as a text to undermine the various theories that have been constructed to explain cancer. But Albrecht by no means lays the dust with his criticisms, while his own hypothesis that a tumor is only a newly developing organ which appears later in life than the normal organs rather thickens the cloud by complicating the conceptions of normal development. We certainly get very little new light on the cause of cancer by giving to the tumor another name or by looking at the abnormal growth as a whole; attention must, rather, be focused upon the underlying biological phenomenon of the division energy of the individual cancer cell.

A half century ago Virchow pointed out the necessity of studying the single cell if we would get at the real interpretation of vital phenomena in health and disease, and biologists and pathologists alike at the present day recognize and act upon the truth of this belief. The single cell is the center of cancer growth; it is the source of metastasis or of transplanted tumor; it is capable of reproducing the disease, and, in short, by its reproduction and life history

*Received for publication January 28, 1908.

[1] Albrecht, E., *Zeit. f. Path.*, 1907, i, 1.

are brought about the biological manifestations which are interpreted as morbid symptoms of malignant growths.

Comparative physiology teaches that while all animals from man to protozoön perform the same vital functions which distinguish animals from plants on the one hand and non-living things on the other, the mechanism through which they are performed becomes more and more simple as we descend the scale. The complicated organs are replaced and the functions are equally well performed by simple organs or by mere tissues in the lower animals. With such simplification in structure the single cells, while individually no larger than the single cells of mammals, have a much more generalized work to perform. The few hundred cells of a hydra or jelly fish, or the twenty-nine to thirty cells of a dicyemid, without aggregation into organs, perform amongst them all the functions of digestion, assimilation, oxidation, secretion, excretion and reproduction, functions which are performed by different organs in higher animals. Such cells are generalized, and physiologically are much more perfectly balanced than are the cells of kidney, liver, heart or brain. Finally, amongst the protozoa, we find cells in which the physiological balance is perfect, for here, in the same protoplasm, are performed all of the vital functions which distinguish animate from inanimate things.

The comparison, then, of a single epithelial mammalian cell with a single free-living protozoön, shows a decided physiological superiority of the latter, since, capable of an independent existence, the protozoön is a much more perfect vital mechanism. The epithelial cell is specialized for the performance of a single function—not to the exclusion of other functions for of course it is living protoplasm, but this one function predominates over all others. Its existence is maintained, its needs supplied, by the activity of other cells similarly specialized for some one function, and it is dependent therefore upon these other co-laborers. The measure of higher animal development is the degree of this differentiation and the complexity is manifested by the fact that these diversified and specialized cells are all coördinated for the good of the organism as a whole. The development of one group of cells is restrained and controlled by this regulating power of the whole, and development

and differentiation find their highest expression in those organisms having the most specialized cells.

In every type of animal there is a more or less well defined cessation of growth when the definitive type is reached. This perfect type development is the goal to which every fertilized egg tends, and to reach it the egg, and the fertilized protozoön as well, are endowed with a certain potential of division energy. When the perfect organism is attained the individual cells cease to multiply and their activities are now directed towards the one physiological object for which they are specialized; division is resumed only when some external cause, such as a wound or other injury, starts up the inhibited development, while even this power of multiplication and regeneration is lost to some types of physiologically unbalanced tissue cells.

Of all cells of the higher animals the epithelial group retain the more perfectly balanced functions longer than those of any other cell type. Here for example belong the covering cells of the body, those of the Malpighian layer of the skin, retaining their division energy throughout the life of the organism, and here belong the germ cells with their potential of endless existence. But even in this group of the epithelial cells the potential of division energy varies, and in the highly specialized and physiologically unbalanced secreting cells it is early exhausted.

It is in the vital manifestations of these cells of the body that we must look for the cause of carcinoma. Here in the dividing cell is the seat of malignant growths, and here in the re-animation of the latent division energy is the real cause of from five to six deaths from cancer in every hundred deaths from all causes.

The carcinoma cell biologically is a perfect vital mechanism. It is no longer an epithelial cell; it has become changed from such a physiologically unbalanced unit, subject to the coördinating control and regulation of the organism, into a physiologically balanced cell, uncontrolled and unregulated.

Structurally the carcinoma cell differs from the functioning epithelial cell type; it is relatively larger; its nucleus is larger, both relatively and absolutely to the cell body, and a reticulated protoplasmic structure shows a marked change from the functional secret-

ing cell. Functionally the carcinoma cell is a more perfect type than its orderly colleagues of the epithelium. It takes in and assimilates abundance of food, grows rapidly, especially when near the immediate source of food, that is, at the growing edge, and reproduces its kind through the same complicated processes of mitosis that characterize free living cells. In short, it is a complete animal organism in itself, simulating in many ways the parasitic protozoön, but differing in some of the most important respects connected with the continued life of the latter.

The long-continued transplantation of the Jensen tumor in mice, the fact that each new transplantation results in the formation of a mass of cancer cells derived from the transplanted cells, and this now through nearly one hundred generations of transplantations, indicates that the cancer cells are somehow endowed with the possibility of an indefinitely continued existence. It is, therefore, different from any animal organism that we know, either among the simply constructed protozoa, or among the more highly organized metazoa, for in all such cases, indefinitely continued protoplasmic existence is bound up with phenomena of fertilization and inheritance quite as subtle and as difficult to analyze as vitality itself. The cancer cell, so far as we know, undergoes no processes analogous to fertilization. The observations of Farmer, Moore and Walker[2] on heterotypical mitosis in cancer cells indicate only evidences of the degenerative changes which the majority of cancer cells must undergo, since it is impossible for all of the products of proliferation to find nutriment in the host organism, or to escape from its protective reactions. Cytologists, furthermore, are constantly demonstrating that heterotypical mitosis is only a condition which may be assumed by cells under abnormal treatment. Haecker,[3] for example, has shown that normal somatic division figures are transformed into heterotypical mitoses by treatment with ether and other poisons, and Miss Bonnevie[4] has recently shown that heterotypical mitoses are common enough in normally developing cells of various animals and plants. This type of mitosis, therefore, has nothing to do with the present problem as evidence at least of the

[2] Farmer, Moore and Walker, *Proc. Roy. Soc.*, 1903, lxxii, 499.
[3] Haecker, V., *Biol. Cent.*, 1904, xxiv, 787.
[4] Bonnevie, K., *Biol. Bull.*, 1907, xiii, 57.

cause of cancer. The further observations of Farmer, Moore and Walker as to a reduced number of chromosomes in cancer cells are better explained along the lines early pointed out by Hansemann, as due to abnormalities brought about by deranged mitotic figures in degenerating cells. So, too, the so-called "fertilization" of an epithelial cell by another epithelial cell, or by a leucocyte, maintained by the English observers, may be easily disproved by any one who takes the trouble to follow out the history of invading cells which are frequently found in cancer tissue. Such invaders disintegrate and ultimately break down into particles which, since the beginning of cancer research, have been variously interpreted as coccidia, as amœbæ, as "X"-bodies, or as "cancer cell inclusions" of one type or another.

Long continued observations on free living cells, such as the protozoa, have shown that periods of activity alternate with periods of depression of vitality in more or less regular rhythmic change. Diagrams I and Ia represent the life histories of two species of ciliated protozoa, *Paramecium aurelia* and *Oxytricha fallax,* the former as worked out by Calkins,[5] the latter by Woodruff,[6] the variations in the curves indicating rhythmic variations in the vitality of the protoplasm under observation. The regularly recurring deep depressions in the *Paramecium* curve represent the periods of complete exhaustion of the division energy, an exhaustion overcome by the use of artificial stimulants. Such studies have proved the unquestionable variability of vitality in these free living forms. In certain embryonic cells, moreover, a more or less similar rhythm in development has been noted by a number of different observers. In this connection Wilson says: ". . . during the cleavage, the individual blastomeres are often found to exhibit entirely different rhythms of division, periods of active division being succeeded by long pauses, and sometimes by an entire cessation of division even at a very early period."[7]

With their balanced physiological activities similar to those of a protozoön, we might expect alternating periods of depression and

[5] Calkins, G. N., *Jour. of Exper. Zool.,* 1904, i, 423.
[6] Woodruff, L. L., *Jour. of Exper. Zool.,* 1905, ii, 585.
[7] Wilson, The Cell, 2d ed., p. 389.

COMPLETE HISTORY BY TEN-DAY PERIODS OF THE DIVISION-RATE OF THE A SERIES.

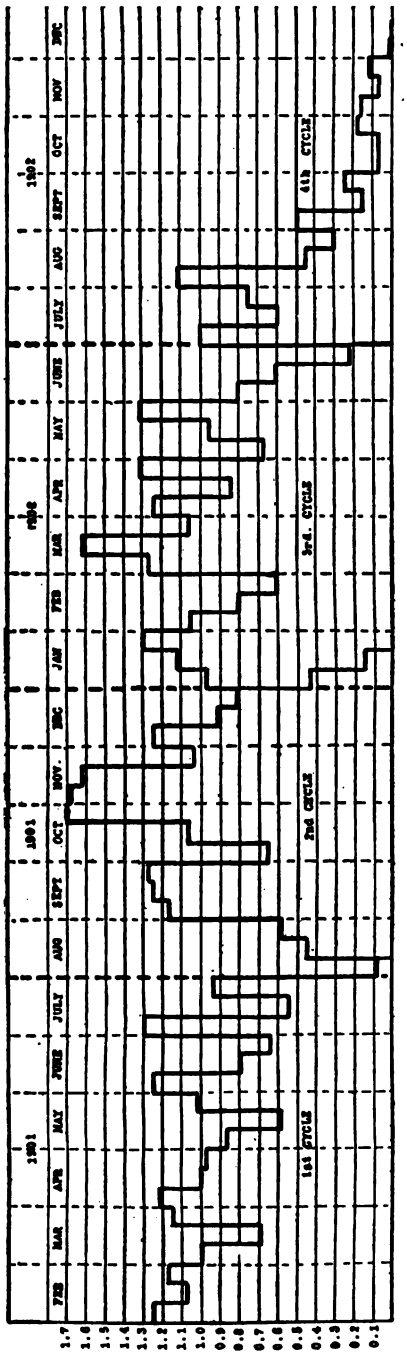

DIAGRAM I.

History of the A Series from start (Feb. 1, 1901) to finish (Dec. 19, 1902) by ten-day periods (three periods to each month). The ordinates represent the average daily rate of division. The heavy dotted lines indicate the limits of the several cycles, and the lines of the curve carried to the base indicate that the individuals that were not stimulated by change of diet died out. The points at which such lines leave the curve indicate the time of the successfully changed diet.

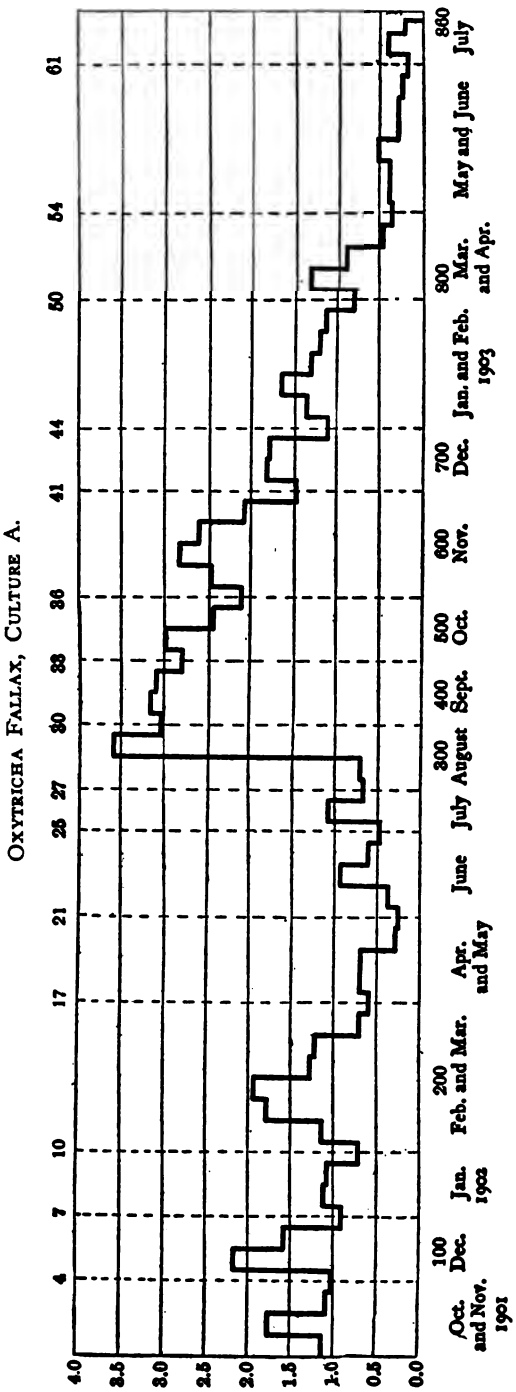

Diagram Ia.

Complete history of Oxytricha fallax, Culture A, from start (October 26, 1901) to extinction (July 14, 1903) in the 860th generation. Rate of division averaged from ten-day periods. The ordinates represent the average daily rate of division of the four lines of the culture. The broken lines designate the limits of the various *rhythms*. Above, the numbers of the ten-day periods which limit the rhythms are indicated; below, the months in which the rhythms chiefly fell. The figures, 100, 200, etc., represent generations and are placed in the periods in which they were attained. (Woodruff.)

of greater activity in the protoplasm of cancer cells. Although the evidence is difficult to analyze because of the many factors involved in the relations of transplanted cells and new hosts, there is a certain amount of evidence to show that alternations of depression and vigor are characteristic of these cancer cells, but whether such alternations are due to variations in the division-energy or to something else remains to be proved.

The evidence for this alternation of depression and vigor in the cancer cell is constantly growing. Bashford, Murray and Boyen have plotted curves based upon the percentages of takes for ordinates and the length of time for tumors to grow to the inoculating point for abscissas. Such curves show alternations or waves of growth and the authors argue that they indicate rhythms of growth energy of the cancer cells.

Such an interpretation, however, cannot be readily granted. In the first place, the rhythms of growth, to be comparable with those of a free living, or of a cleavage cell, should be looked for in the individual mouse and not in successive batches of mice. In the second place a curve such as Bashford, Murray and Bowen[8] present, introduces two distinct factors, one is the percentage of "takes" on transplantation which I will call the "infectivity" of the cancer cells, the other is the time factor in development of a tumor. It is this time factor which really measures the growth energy of the cells, the length of time required for a tumor to develop indicating roughly the rate of division of the cells. With the statistical evidence at hand these two factors may be compared, and the comparison shows that something besides the growth energy is involved in what is usually regarded as the "malignity" of cancer cells. The accompanying diagram illustrates the point I wish to bring out.

In this diagram the one curve (continuous line) represents the variations in infectiveness of one of the Buffalo Laboratory tumors, known as the Brooklyn tumor; the other curve (broken line) indicates the number of days required for development to the point necessary to kill the inoculated mice. Each curve is based on averages of all mice involved in the successive transplantations, and the points obtained in each curve represent common transplantations, so that percentage of takes and length of time refer to the same

[8] Bashford, Murray and Bowen, *Proc. Roy. Soc.*, 1906, B, lxxviii, 195.

cancer cells. For example in Period Number 4 the tumor cells upon inoculation in a large batch of mice gave tumors in 39 per cent. of the mice; a large number of these (17) died from the tumor, the average length of time required being sixty-six days. Here, then, is a means of measuring the relative growth energy of the cancer

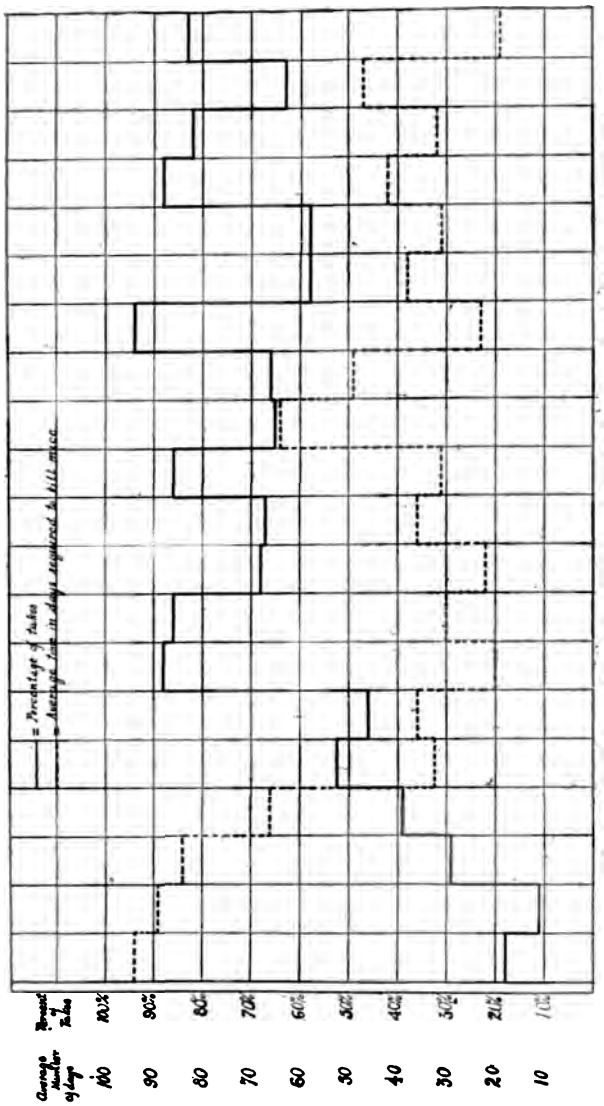

DIAGRAM II.

cells at different periods and a corresponding measure of the infectiveness of the same cells. The curve shows that the two factors are by no means equivalent and that too much is assumed in choosing the percentage of takes as the measure of growth energy. At the outset and in the early transplantations there is, it is true, a definite relation between the energy of growth and the infectivity: the number of days required falls steadily and the percentage of takes rises just as steadily, but after the tumor is fully established the relationship apparently disappears and the two curves behave differently, the infectivity remaining high on the average and quite independent of the growth energy as represented by the time factor. The waves of growth described by Bashford, Murray and Boyen are here shown by the regularly recurring alternations of infectivity, and not in the line showing growth energy.[9] It is the factor of infectivity, therefore, which Bashford, Murray and Boyen have interpreted as the factor of growth energy. It is this factor in transplanted cancer that runs in rhythms in successive batches and not the growth energy alone. The same phenomenon of contrast is shown in a curve (Plate XX) for our Brooklyn tumor similar to that which the English pathologists give for the Jensen tumor as observed in their laboratory. For this curve the percentages of "takes" are used for the ordinates and the number of days required for the tumor to grow to the inoculating point, for the abscissas. The several points on the curve marked with letters and numbers indicate the successive transplantations of the tumor cells. The relative time of development is shown by the length of the lines between the points, while the successive waves represent the rhythmic alternation of the infectivity. This curve has the appearance of being stretched out at the ends and concentrated in the middle, a feature demonstrating the same phenomenon as the double curve in Diagram 2. It is based upon somewhat different statistics, the abscissas representing the number of days from inoculation to inoculation instead of from inoculation to death of the mice inoculated as in Diagram 2. The longer lines at the beginning and the end

[9] The minor fluctuations in the line showing growth-energy, although quite regular in this series, are so small as to fall within the limits of error. In similar curves for other tumors this regularity is not evident, while the characteristic rhythms shown in the curve of infectivity are similar to those here given.

of the curve show comparatively long periods of growth, *i. e.*, relatively little growth energy, while in the center the shorter lines mark a more rapid growth. After the initial period of the first three or four inoculations the percentage of takes rises and remains high quite regardless of the variations in number of days required, *i. e.*, regardless of the growth energy. The same rhythms noted by Bashford, Murray and Boyen are evident here, sometimes concentrated, at other times drawn out according to the changing abscissas.

The tumor upon which this diagram is based was treated as all of our tumor material for transplantation is treated and a description of the process will give the basis of our belief in the specific division energy of the individual cancer cells. The original tumor cells, and all succeeding tumors, were ground up in a mortar with a given volume of salt solution. The connective tissue strands were removed by raking, and uniform doses of one half a cubic centimeter were introduced subcutaneously into each mouse of a series.

This method of transplantation means that the cancer cells are separated as far as possible from one another which gives a greater chance for individual cells to respond, or, on the other hand, to be acted upon by the protective factors of the host. Their response, that is, the tumor which develops, is, perhaps, a better indication of the division energy of the cells than if a large piece of the cancer tissue is introduced. The rate of growth of similar amounts of such cancer cells inoculated at successive transplantations of the tumor, gives a reasonably accurate measure of the relative infectivity of the cancer cells at different periods, and this variable may be charted.

Transplantation (1) of the primary tumor was made May 30, 1905, the cancer mush being put into 28 mice. None of these died inside of ten days, while 5, or 18 per cent., developed tumors. One tumor suitable for transplantation developed in 75 days, while 2 of the cancer mice died. This tumor (2) was similarly teased and the cancer mush inoculated in the regular dosages, into 30 mice, of which 3 died inside of ten days and 3, or 11 per cent., developed tumors. One of these tumors (3*a*) was ready for further inoculation in 53 days; a second (3*b*) and a third were ready in

66 days. These in turn were inoculated into different lots of mice of the fourth transplantation series, that lot inoculated with *3a* giving 33 per cent. of takes, and that from *3b* giving 25 per cent. The tumors from this second lot were used for further transplantations, the former for experiments and are therefore unsuitable for the present discussion. The mouse tumor designated *3b,* giving 25 per cent. of takes upon re-inoculation, developed four tumors which were ready for transplantation in 39, 51, 53 and 64 days, and they were inoculated in different batches of mice of the fourth series.

With the three transplantations which had been made up to this point the cancer cells had been growing from 180 to 200 days, and in this time there is a noticeable shortening of the period of development from 75 days after the first transplantation to 39 days in the case of *4a,* and 53 days in *4b.* The increasing percentage of takes, combined with the shortening of the time of development, indicates that the cancer cells were becoming accustomed to the soil, to use Bashford's expressive term, of the different host mice, and were gaining strength with each new transplantation. That they were not yet perfectly adapted, however, is indicated by the fact that the tumors which developed upon the fourth transplantation were not equally established, some dying out altogether within two subsequent transplantations, while others gave rise to highly virulent cancers. Thus *4c* inoculated into a batch of mice produced only 18 per cent. of takes (*5b*), and these required 41 days, while the same strain upon re-inoculation into the sixth series, produced only 4 per cent., after which the strain died out entirely. Tumor *4b* had a more remarkable history for some of its cells were apparently endowed with a very high, and others with a very low, potential of vitality. This tumor, upon inoculation into the fifth series, produced only 15 per cent. of takes (*5f*), a marked decrease, but one of the tumors which developed in a short period of 23 days (*5f*), gave rise to a most malignant strain running up to 100 per cent. of takes in the twelfth transplantation (*12a*), while another tumor (*5c*) from the same source (*4b*) required 64 days and was comparatively feeble, for, upon re-inoculation into the sixth series, it gave only 4 per cent. of takes. It is seen, therefore, that at this early period of transplantation, the same tumor may give rise to a

highly virulent strain, or to a weak strain, showing that the cancer cells are not fully adapted to new soils. Of course there are always two factors to take into consideration, one is the division energy of the cancer cells, the other, the natural resistance of the host into which such cells are inoculated. It is not improbable that the batch of mice into which *4b* was transplanted had a relatively high protective reaction, so that the division energy of the cells as a whole was restrained, but the rapid growth of *5f* shows that here was a mouse in which no such restraint was manifest, the natural resistance must have been weak or else the particular nutritive conditions must have been suitable, for the cancer cells acquired a high potential of activity. Tumor mouse *4d* must have been similar to *5f* in this respect, for the same cancer cells upon re-inoculation into the fifth series, now gave 80 per cent. of takes (*5d, e*), as against 15 per cent., and in periods ranging from 34 to 42 days.

The further history of the Brooklyn tumor is the working out of these two lines of development, one from *4d*, the other from *5f*, and in both strains the characteristic rise and fall in rhythmic change is well shown in the curve. The high percentage of takes from *4d* is continued in the next transplantation, *5d* alone being used as the others were taken for experiments. This tumor, upon inoculation in the sixth series, gave 77 per cent. of takes in the short periods of 25 (*6b* and *c*) and 43 (*6a*) days. After this, however, the infectivity declined for these tumors upon inoculation into the seventh series, yielded only 50 per cent. (*7b*) and 33 per cent. (*7a*) in 33 and 36 days, respectively. The latter (*7a*) upon re-inoculation, however, shot up again to 80 per cent. in the eighth series and in 26 days, thus marking a definite rhythm.

The history of tumor *5f* is more interesting and is marked by a series of rapid growths and by a gradually increasing percentage of takes. It was transplanted into 9 mice, of which 2 died in ten days, while in the others 2, or 28 per cent., developed tumors. One of these died from the cancer; another (*6c*) had a transplantable tumor in 18 days, and this, when transplanted into 20 mice, yielded 65 per cent. in 20, 39 and 80 days. In the latter case (*7d*) there is considerable evidence to show that the tumor was held back by the resistance of the mouse host, for the malignancy of the same tumor material was shown by the rapid growth of *7c* and by the

fact that *7d*, when transplanted into a new batch, gave rise to 77 per cent. of takes and all within 28 days (*8b, c*).

At this period in the history the curve becomes complicated through the converging of lines but they all show a certain optimum of development followed by a fall and this by a renewal of growth. The tumor *7c* is a good average example; this, when transplanted into series eight gave 88 per cent. of takes, some (*8c* and *8x*) in 21 days, one (*8b*) in 15 days and one (*8x*) in 26 days. None of the mice inoculated died within ten days. The history of this set of tumors varies somewhat in the different cases; in some the division energy of the cancer cells decreased immediately, in another it decreased only after the following transplantation. Following out the history of *8c*, we see from the diagram that, upon re-inoculation into a fresh batch of 11 mice, none died within ten days while 7, or 80 per cent., developed cancer, *9d*, in 13 days, *9e* in 24, two others in 38, and two in 41 days. Many of these were used for experiments, but two of the rapidly growing ones were transplanted (*9d* and *9e*). In both cases there was a marked decrease in the division energy of the cancer cells: *9d*, which had developed very rapidly (13 days), was transplanted into 6 mice of Series 10, one of which died within ten days, while only two tumors, or 33 per cent., developed, these requiring 25 days. One of these two (*10e*), upon re-inoculation into fresh mice of Series 11, gave rise to 61 per cent. of tumors, two of which (*11f* and *11g*) were used for experiments, the first developed in 29 days, the second in 77.

Turning back to *9d*, which had the same ancestry as *9e*, there is the same evidence of depression in the energy of the cancer cells coming from *8c*. Tumor *9d* was transplanted into 16 mice, of which 6 died within ten days, while 66 per cent. developed tumors, of which the greater number were used for experiments, all developing within 31 days. One (*10a*) was used for further transplanting into Series 11; it was ready for inoculation in 19 days, and was put into 26 mice of Series 11. Of these 4 died within 10 days, while 10, or 43 per cent., developed cancers, *11c* in 18, *11a* in 30 days. *11a* was inoculated into 10 mice, of which 8, or 80 per cent., developed tumors. Its history, therefore, is identical with that of *10e* except that it was one generation behind the latter. There is

the same lull in the division energy and the same recovery, and the same rhythm in infecting power.

All of the tumors described are to be traced back to tumor 8*c*, which came from the cancer cells in 7*c*. A very similar history is shown in the case of tumors coming from 8*b*, likewise derived from 7*c*. Here again the general result is a high percentage of takes with short periods of growth, or in general, a high virulence of the cancer cells. 8*b* was developed and ready for transplantation in 15 days when it was put into 20 mice of the ninth series; none of these died within ten days, while 18, or 90 per cent., developed tumors, many being used for experiments of one kind or another, while four were transplanted into batches of the tenth series. The periods of development of the 13 tumors were for the most part relatively short; 9*a* and 9*x* requiring only 14 days, two others 18 days, 9*b* and 9*c*, 20 days, three others 23, others 30, 34 and 44 days, respectively. Of these 9*a*, 9*b* and 9*c* were used for further transplantation and their histories are remarkably similar. 9*a*, when inoculated into mice of the tenth series (10*d*), gave 75 per cent. of tumors which required from 25 to 40 days to develop, and these tumors were used for experiments. 9*b*, when transplanted, gave a small percentage (43 per cent.) of takes (10*f*). There was, therefore, a well marked decline in the division energy of the cancer cells and the one tumor required 28 days to develop and this, when transplanted into another batch of mice, caused their death inside of ten days. Of this ninth series, the tumor marked 9*c* was the most successful and also the most remarkable; it was transplanted into 30 mice of the tenth series, of which 3 died within ten days, while 20, or 80 per cent., developed cancer in from 14 to 27 days, all rapidly growing tumors, two of which (10*b* and 10*c*) are given in the curve, the former developing in 14 days, the latter in 20. Each of these tumors, when transplanted into new lots of mice of the eleventh series, gave reduced percentages of takes. Tumor 10*b* fell to 62 per cent., 11*b* and 10*c* to 70 per cent. The progeny of 10*c* were used for experiments, while 10*b* was used for further transplantation. It was put into 24 mice of the eleventh series, of which none died, while 15, or 62 per cent., developed tumors as follows: 11*b* in 7 (sic) days, another in 24, 11*d* in 26, others in 27, 29 and

32 days, respectively. Of these tumors only 11*b* and 11*d* were used for further transplantation, the others going into experiments. Here, however, 11*b* is of especial interest, for upon inoculation into 6 mice of Series twelve, 100 per cent. developed tumors (12*a*). Of these, one recovered spontaneously, while the others, when inoculatd into new batches of mice, caused the death of all the latter within ten days (?). This malignant strain was thus brought to a speedy end.

The other strain from 10*b*, carried in mouse 11*d*, had a better fate. It was a more slowly growing tumor, requiring 25 days to develop. This tumor, upon transplantation in 16 mice of Series twelve, gave rise to 94 per cent. of takes in from 20 to 28 days. Most of the resulting tumors were used for different purposes but one, 12*b*, which required 25 days, was again transplanted into 30 mice of Series 13, one of which died and 65 per cent. developed cancer in periods varying from 12, 14, 18, 23 and 31, to 94 days. Most of these were not followed up, one only, 13, which required 31 days, was transplanted into 8 mice of the fourteenth series, giving 66 per cent. in long periods of development (56 to 69 days).

Anyone who follows through these tedious details will see that the cancer cells contained in the tumor-bearing mouse, 7*c*, all had the same history of high proliferative energy followed by a decline. In all cases followed out, this decline is followed by a sharp rise in division energy and in the percentages of takes. Sister cells of tumor 7*c* formed the tumor in 7*d*, but required a much longer period (80 days) to develop. Although slower, these tumor cells had a high potential of division energy, as shown by the fact of high percentages and quick periods in the eighth and ninth series. The tenth series, like the tenth series in the allied strain, brought a decline, thus marking for this strain a similar rhythm of growth energy.

The further history of the transplantations needs no special analysis; as the curve clearly shows, there is a continuation of the rhythms of infectivity, the average of which remains high, while the time element becomes much longer, until the curve now has the aspect of having been pulled out at the ends and concentrated in the middle.

It is hardly conceivable that this rhythmic development of the cancer cells is only artificial, due, that is, to the fact of inoculation into particularly susceptible mice, of which, according to Bashford, young mice offer the best examples. Such an explanation might be satisfactory for one or two instances of the phenomenon, but recurring regularly as it does, the phenomenon probably signifies something fundamental. In a developing embryo the rhythms of growth energy of the individual cells do not indicate a cessation of growth for the organism as a whole, for while some of the cells are resting others are dividing and developing. The energy of growth is applied sometimes in this sometimes in that group of cells. In cancer, however, there is only the one type of cells to develop, and appearing in different batches of mice the phenomenon cannot be attributed to the source of nutriment of the cancer cells, but must be looked for in the cells themselves. As previously stated these cells must be regarded as complete organisms, satisfying all of their physiological needs through their own physiologically balanced activities. The source of the alternating rhythms must, therefore, be sought in the cells. In other and possibly analogous cases of complete cellular organisms the cause of the alternating periods is looked for in the variations of the physiological activities. Thus in the *Paramecium* experiments already mentioned, high division energy of the individual cells is followed by a gradually lowering rate of division until it is reduced to nil. At such periods of depression it was found that the cells could not reduce the highly stable condition of the protoplasm to a more labile condition, and that they evidently lacked the power of oxidation, or of forming the oxidative enzymes. This condition was relieved by the use of certain salts acting as stimuli; by them the labile condition was reëstablished and divisions in rhythmic waves of vigor again ensued (see Diagram 1).

In the *Paramecium* cycle there was nothing to interrupt the dividing activity of the organisms save the ordinary course of vitality. With the cancer cells there is always the struggle to maintain an existence against what must be adverse conditions. The division energy of the cells is retarded by such conditions, a result best shown in the early periods of the transplantations before the cells became

adapted to the strange soil. The same phenomenon is manifest in the culture of *Paramecium,* a few days being required for the cells to become adapted to the culture medium. They seem to survive well enough in the medium, but they do not divide with such regularity during the first few days as afterwards when well established. The difference is due to the density of the fluid medium in which the organism finds itself rather than to any change in food. And so it may be with the cancer cells; they have the same source of food in the new mouse to which they are transplanted, but the environment is a little different and it requires some time for them to get started. Once started, however, there seems to be but little change in their potential of infectivity, although the periods of development differ to some considerable extent, the difference probably being due to the protective reactions on the part of the host mice. This is shown well in Diagram 2, where the periods of development in days are contrasted with the percentages of takes in the different transplantations from start of the tumor to the present. The plotted curve shows that as the number of days required for development in the successive periods decreases, the percentage of takes increases with great regularity, until, when once established, it is maintained regardless, apparently, of the length of time required for development. This fact indicates that the cancer cells are endowed with a like power of infection, but that the host animals exert a retarding influence upon their development. All cancer cells cannot be expected to develop, hence it is too much to expect 100 per cent. of takes all the time; those cells maintain their existence which are endowed in the parent tumor with the most advantageous equipment for growth. This is not to be expected in imperfect cells, or in degenerated cells, but in those having the full complement of cell organs, normal number of chromosomes, division energy and the like. The success of such cells may be compared with that of the gametocytes of the malaria organisms; these are endowed with some property through which they are able to withstand the digestive fluids of the mosquito's gut, while all other forms of the malaria organisms are digested. So with the cancer cells those persist which have the proper attributes and these attributes are apparently developed with some degree of periodicity.

Is there any evidence to indicate the nature of these particular attributes?

The cancer cell gets its nourishment as do other epithelial cells; there is little evidence to support the belief of Ehrlich that they make use of a specific *Nahrsubstanz,* although they are undoubtedly so modified as to take up more nourishment. They are bathed by the same fluids, and are subject to the same general conditions of metabolism as other epithelial cells. Other epithelial cells, however, do not multiply beyond the limits of organic regulation; these cells do and they are cancer cells because of this power. Other normal epithelial cells, beyond one or two divisions, do not multiply upon transplantation into other mice. The cancer problem, therefore, has to do with the specific attribute which distinguishes the cancer cells from these other epithelial cells, and we are again brought back to the question—what is the origin or source of the stimulus which causes this infectivity combined with the increase of the division energy? The conditions of the environment of cancer cells in new batches of mice cannot account for the stimulus to continued development in successive transplantations; if this were true the percentage of takes and rapidity of growth would be quite as extensive on the first transplantation as in the later ones. This, also, would be the anticipated result if the cancer cells have that vigor of growth which should accompany them if they were embryonic tissue cells, or cells recently stimulated by some initial stimulus, or cells recently fertilized. The cancer cells must carry with them the sources of their stimuli, and these stimuli must be the same, both in the primary tumor and in the continued development of that tumor's cells, for we cannot conceive that each new batch of mice into which the cancer cells are transplanted provide the same identical physiological conditions as those of the first mouse in which the cancer was a primary tumor. Furthermore we cannot conceive any reason why, if the proper physiological conditions were present, there should be rhythmic variations in their production in different sets of mice, hence the conclusion seems inevitable that the source of the stimulus to development is within or associated with the cancer cell itself.

In the entire realm of biology there are few analogues to this

condition of affairs. The favorite hypothesis of the medical profession,—the Cohnheim theory of embryonic tissue rests as the cause of cancer, cannot stand the biological test. Leaving out of consideration that entirely unexplained set of questions having to do with the position and conditions of such cells during the periods of growth and maturity, the cancer cells, after the tumor is started, have none of the attributes peculiar to embryonic cells. Mere power of proliferation is not a sufficient characteristic, while the fundamental characteristics of embryonic tissue cells are usually entirely ignored by the advocates of this theory. These conditions are somewhat more subtle than are the ordinary manifestations of vitality, but they are none the less potent. I refer to the properties which all embryonic cells have of differentiation combined with coördination and self-regulation. Cancer cells have none of these; there is no evidence of differentiation although mountains of mouse tissue have been grown from the cell progeny of the original Jensen mouse. The absence of any regulation and of all coördination and of adaptation to the needs of an organism as a whole which is the goal of all embryonic cells, is evidence against the hypothesis. All of these factors are characteristics of embryonic cells, and to the biologist they are the most important and the most characteristic. No theory of embryonic tissue satisfies the conditions of increased vitality and vigor of growth shown by this Jensen tumor, and we must look in other directions for its origin.

Nor can the tumor origin be traced to any detached cell, which, freed from its supporting and regulating membrana propria, starts off on an independent career of lawless development. This theory of Ribbert postulates the metamorphosis of an ordinary epithelial cell into a parasite endowed with a potential of indefinite vitality and development. It does not account for the origin of the stimulus to the latent division energy, much less does it account for the perpetuation of that stimulus. Here, too, lies the difficulty with the theory of chemical stimulation which Marchand has elaborated. There is no evidence that an initial stimulus can continue to act in this direction indefinitely, the impetus given soon wears off, and the gradually increasing vigor of a tumor like that of the Brooklyn mouse, is evidence quite strong enough to indicate that the stimulus, whatever it is, acquires strength rather than loses it.

The evidence, finally, as I interpret it, shows that the cancer cell carries with it into each new mouse into which it is transplanted its own stimulus to division. Furthermore this stimulus presumably issues from a foreign organism rather than from an integral part of its own protoplasm, or from a product of its own metabolism; the very definition of a stimulus involves the idea of an external origin. We are thus driven back by these considerations and by the facts of infectivity as shown above, to the last resort of a biologist or pathologist, viz., to the suggestion that *the cause of the division energy lies in the stimulating poison from some self-contained and ordinarily invisible micro-organism whose rhythmical variations in vitality may, possibly, account for the rhythmic variations in infectivity.*

In this connection we find one of the very few analogues of cancer cell growth, the formation of a vegetable gall where the latent division energy of the plant cells is stimulated to renewed activity by a poison secreted by an insect. As often pointed out, however, this is only a far-fetched analogy, for the stimulus comes to an end, and the division energy wears off. A more significant case is the formation of tumors on the roots of certain plants like the cabbage and its allies. Here, in club root, the parenchyma cells of the root are entered by a minute protozoan parasite. The organism multiplies in the cell protoplasm, which reacts to the stimulus of the parasite's presence, and divides. The division is repeated until great tumors are formed, parasite and protoplasm meantime living amicably together in symbiosis.

As all attempts to explain the cause of cancer must at the present time be of the nature of tentative or working hypotheses, the suggestion of an ultra-microscopic or unrecognized organism is not out of place, the value of an hypothesis being measured by the number of phenomena which it accounts for. The old arguments in favor of the parasite theory of cancer, familiar to everyone conversant with the subject, apply in the present case. Cage infection, early noted by Borrel, observed by Giard, Ligniéres, Michaelis, in the Buffalo laboratory, etc., finds its interpretation in such an hypothesis. The infectivity of cancer cells, a phenomenon by which these cells differ from ordinary epithelial cells treated in the same

way, is added evidence which finds a counterpart in the tumors of club-root type. Contrasted with these are tumors like vegetable galls, certain benign tumors and embryonic tumors of man, in all of which there is a well-defined division energy of the cells involved, but no infectivity on the part of these cells. In club root and in cancer, division or growth energy is accompanied by clearly defined infectiveness. In club root infectivity is due to a contained parasite, and the growth energy is attributed to the stimulus from that parasite.

In cancer we have found no parasite confined exclusively to cancer tissue, but analysis of the growth energy and the phenomena of transplantation give evidence to show that infectivity and division energy of the cancer cells are not one and the same thing and that each has its own variations. The growth energy or division energy of the cancer cells, measured by the periods required for growth in successive batches of mice, affords no evidence of rhythmic development such as we find in embryonic cleavage cells or in free living organisms like the protozoa; there is, in all probability, such a rhythmic growth but it must take place in the individual mouse if at all. The infectivity, however, shows a regular rhythm which cannot be attributed to the reactions of mice in batches or lots of varying number, but this periodicity may be explained as the regular variations in vitality, similar to those of paramecium, oxytricha or tillina of a parasitic organism.

Many parasites have been assigned to the human cancer cells. Protozoa of all types have been held responsible by one or more investigators, but in no case have the claims been made good. The various morphological products of epithelial or blood cells that have been called parasites have served only to weaken the hypothesis of the parasitic origin of cancer, which, with its power of indefinitely continued proliferation is a very different thing, biologically, from a benign tumor.

It is certainly conceivable that a parasite of cancer may be too minute to be seen with the technique at our disposal. At the present time we know a great deal about the yellow fever organism; we know the period of incubation it requires in the human blood; we know that it requires from twelve to fourteen days to develop in

the body of a mosquito before the latter is able to transmit the disease; we know that the disease cannot be transmitted in any other way, and yet, knowing all of these things, the organism of yellow fever has never been seen. It will pass through the finest filters, and belongs, therefore, to a group which we must perforce, until they are actually seen, consider as ultra-microscopic organisms. Such parasites might be adapted to life in the epithelial cell as well as the organisms of club root are, and there in the protoplasm might easily be overlooked. It has been suggested that a species of *Spirochæta* is responsible for yellow fever, and spirochætes have actually been seen in the kidney of yellow fever victims. But they apparently do not exist as such in the blood or in the mosquito. We know nothing about the life history of the spirochætes as a group; if it is analogous to the life history of most protozoa we might well look for stages in which it is of ultra-microscopic size.

We continually find spirochætes in mouse cancer and it is not improbable that we attach too little importance to them. We find furthermore a certain relation between the virulence of mouse cancer and the numbers of spiral organisms present; in highly virulent strains the tissues are infested with the parasites and it is not beyond reason to assume that the rapid death of batches of mice inoculated with virulent cancer cells is due to these spirochætes. Nor is it inconceivable that the parasites increase the susceptibility or prepare the soil, so to speak, in a new host for the growth of the cancer cells. On the other hand, the same or a very similar spirochæte is found in mice not infected with cancer, a fact which weakens any theory that the spirochæte is the direct cause of cancer, although the fact is not fatal to such a theory. A more serious objection is that no spirochætes are found in human cancer, but the objection might be met with the argument that spirochætes may have been present at the outset to prepare the soil and to provide the stimulus to the potential dividing energy; furthermore, we do not know that human cancer is transplantable. The rhythms of infectivity, finally, may be due to the variations in vitality of these spirochætes.

SUMMARY.

In conclusion we may summarize the above biological obs tions in a series of theses as follows:

1. Cancer cells differ from other epithelial cells in respec (*a*) Size relations of nucleus and cell body; (*b*) power of in nitely continued division.

2. Cancer cells differ from embryonic cells in absence of: power of differentiation; (*b*) power of coördination of part whole; (*c*) power of self-regulation and limit of growth.

3. The continued development of the cancer cells is subject to following factors: (*a*) the inherent potential of division of cancer cells. (*b*) The natural resistance of the inoculated anim The latter factor is usually regarded as the index of malignancy a tumor and is based upon the percentage of takes together with period required to kill the mice. Our experiments, however, sh that the percentage of takes is independent of the time factor, a indicate the presence of a third factor which may be described (*c*) the potential of "infectivity" of the cancer cells.

4. The potential of infectivity of cancer cells is characterized more or less regular rhythms; these must be distinguished fro rhythms of growth energy of the cancer cells which in all prob bility occur within the individual mouse. Without the divisio energy of the cancer cell this infectivity is inoperative, hence follows that the cause of the infectivity lies within the cancer ce or is constantly associated with it.

5. Cancer cells differ from epithelial cells by virtue of this poten tial of infectivity combined with that of division energy. There is reason to believe that the latter is due to the action of stimul and not to the liberation of a restrained growth power of embryonic tissue. There is reason to doubt that an initial and discontinued stimulus is responsible for these attributes of the cancer cells. Certain benign tumors, or vegetable galls, may be due to the action of such initial stimuli, but in them there is no infectivity. Embryonic tumors, due to embryonic cells, have a high power of differentiation combined with their division energy, but there is no infectivity. Infectivity distinguishes all cancerous growths from normal epithelium and from benign tumors or teratomata.


13
11
11
400

6. The rhythms of infectivity of cancer cells, erroneously regarded as rhythms of growth energy by Bashford, Murray and Boyen, appearing as they do in successive batches of mice which we may legitimately assume to have like powers of resistance, must have their cause in the cancer cells themselves. These cells, therefore, must be equivalent to parasites, or else parasites are contained within or associated with them.

7. Upon any other hypothesis it is difficult to conceive of cells creating a continual stimulus to their own growth energy, and it is still more difficult to explain the rhythms of infectivity.

8. Many lines of evidence point to the presence of some possible organism within the cancer cell; some organism which, acting as does *Plasmodiophora brassicæ* within vegetable cells, underlies the infectivity of cancer cells and provides the stimulus for their continued proliferation. Upon such an assumption the numerous cases of cage infection find their explanation.

9. The various inclusions of the cancer cell which have been described as organisms have been disproved; yet the analogy of club root and the many filter experiments show that the cause of infection may lie within the cancer cell. It is conceivable that, like the yellow fever organism, such an incitant may be in the protoplasm and beyond our powers with the microscope to locate.

10. The spirochætes which we have found in mouse cancer may have something to do with this infectivity of cancer cells. They may be useful in preparing the "soil" in new mouse hosts and making it susceptible to cell growth; or they may have intracellular stages in their life history which are too minute to be seen. The rhythms of infectivity, finally, may be an expression of the vitality of these spirochætes or of the hypothetical ultra-microscopical organisms accompanying cancer cells.

A CONTRIBUTION TO THE PATHOLOGY OF MYASTHENIA GRAVIS. REPORT OF A CASE WITH UNUSUAL FORM OF THYMIC TUMOR.[1]

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PLATES XXI–XXIII.

Since the publication by Wilks in Guy's Hospital Reports for 1877 of a case presenting the clinical features of bulbar paralysis, yet without any demonstrable lesions at autopsy, our clinical knowledge of this condition has been enriched by the publication of a fairly numerous series of similar cases. Various mental, sensory, secretory and vasomotor symptoms have been added from time to time by different observers to the motor phenomena that usually dominate the clinical picture. Most characteristic among the latter are extreme muscular weakness and the myasthenic reaction first described by Jolly in 1895. Jolly found that by subjecting the affected muscles to a tetanizing faradic current at intervals of one or more seconds the muscular contractions became progressively weaker, until a paresis or even paralysis resulted. The same effect follows repeated voluntary contractions of any group of affected muscles. In either case a short period of rest is followed by a return of the muscles to their former condition of excitability. Not less characteristic are intercurrent attacks of dyspnea and tachycardia with or without fever, symptoms pointing to an apparent bulbar affection. Of the many names proposed for this symptom-

[1] Presented at a meeting of the New York Neurological Society, March 3, 1908.

complex, that advanced by Jolly, "Myasthenia gravis pseudoparalytica," is most generally accepted.

Pathologically our knowledge of this condition is far less complete. A review of the literature prior to 1901 is contained in Oppenheim's exhaustive monograph on the subject. We have been able to collect from the literature forty-five cases with more or less complete autopsy records. To this number we desire to add the following case that seems to present some features of especial interest.

P. C., aged 52 years, a tailor, Russian by birth, was admitted to Mt. Sinai Hospital on December 15th, 1906, to the service of Dr. B. Sachs, to whom we are indebted for the clinical history and use of the case for publication.

Family History.—Negative.

Past History.—Had measles in childhood. Uses alcohol very sparingly, but is an excessive cigarette smoker.

Present History.—Six months prior to admission, after exposure, patient experienced a dull heavy sensation in chest together with difficulty in inspiration. At the same time he noticed diplopia and diminution in acuity of vision that glasses failed to relieve. During the past month vision has improved, but diplopia persists. There has been lachrymation for the past week. Very shortly after the onset, impairment of motion of shoulder girdle on both sides as well as atrophy of the affected muscles developed. Two months later, with impairment of the movements of the tongue, there ensued difficulty in phonation, and regurgitation of food through the nose. For the past three months there has been gradual atrophy of the facial muscles with difficulty in swallowing. The head has a tendency to fall forward on the chest. Patient complains of severe frontal headache, tinnitus aurium, and disturbance in senses of taste and smell. There has been marked emaciation and general weakness.

Physical Examination on Admission.—General condition fair. Moderate emaciation. Sensorium clear. Hearing, taste and smell normal.

Eyes—Pupils contracted, but reaction to light and in accommodation normal. Slight lateral nystagmus. Corneal reflex absent. Slight weakness of external recti. Very marked weakness of levatores palpebræ and of orbiculares palpebrarum. Diplopia at a distance of five feet. Color vision slightly disturbed.

Mouth—Tongue deviates slightly to left. Slight coarse tremor; no atrophy. Pharyngeal reflex diminished. Articulation indistinct.

Face—Flattening and falling in of cheeks. Jaw movements normal. Cannot purse lips, but can retract them to show teeth. Wrinkling of forehead normal. Jaw jerk and Chvostek's phenomenon present.

Lower Extremities—Patellar, Achilles, cremasteric and superior epigastric reflexes normal. Muscular power fair. Gait shows weakness but is otherwise normal. Romberg's symptom absent.

Upper Extremities—Movements of flexion and extension at wrist joint normal; at elbow weak. Range of motion at shoulder joint unimpaired, but all voluntary movements are weak, and even the slightest counter pressure can not

be resisted. Slight atrophy of both deltoids and of infra- and supraspinati. Repeated voluntary abduction of the arm is followed by complete transitory paralysis of the muscles. Ataxia, fibrillary twitching and hypertonicity are absent in the muscles of all the extremities. There is no reaction of degeneration, but a typical myasthenic reaction can be elicited by repeated stimulation with a tetanizing faradic current in the deltoid, spinati, and thigh muscles.

Neck and Trunk—Head held erect with difficulty, and for a short time only. Patient can not assume the erect from the recumbent posture unaided.

Thoracic and abdominal organs negative, except for a mild grade of pulmonary emphysema.

Urine shows a few granular casts; no albumin. Blood count normal.

From January 20 to January 23 there was a gradual but slight improvement in the patient's condition. The head could be raised from full flexion and maintained erect. Voluntary movements of the arms were performed with less fatigue, and the power of resistance to counter pressure was increased.

On January 24 it was noted that without premonitory symptoms, the pulse rate suddenly increased to 104 and the respirations to 70. Breathing was stertorous, but the pulse remained of good quality. Two hours later coma supervened. This condition lasted two hours, after which the symptoms partially abated in severity, the pulse, however, becoming irregular, and the dyspnea continuing. The following morning a similar attack occurred, death ensuing within five minutes of its onset. The course of the disease was afebrile throughout. A report of the autopsy performed twenty-four hours after death by Dr. E. Libman follows:

Autopsy.—Marked rigor mortis; well nourished. Lipomatosis of mesentery. Large lipomatous mass in falciform ligament.

Muscles—All skeletal muscles look pale.

Thyroid—Not enlarged; no macroscopic changes.

Thymus—Weighs 20 grammes; very firm; measures 5 cm. in length, 3.5 cm. in width and 2 cm. in thickness. On section is pinkish-white in color, very firm, lobulated, and cells can be very easily scraped from surface.

Trachea—Negative.

Lungs—Old adhesions over both lungs. They are intensely congested. Small old cheesy area at right apex. Bronchial lymph nodes and mediastinal nodes are anthracotic, some fibrous, others containing small cheesy areas.

Heart—Right auricle dilated. Tricuspid segments slightly thickened. Slight fatty overgrowth over right ventricle. Left auricle dilated. Mitral segments somewhat thickened. Chordæ tendineæ thickened and somewhat shortened. Aortic flaps thickened at insertion, slightly retracted. Corpora arantii thickened. Anterior coronary artery shows fairly marked thickening and atheroma at its origin. No congenital lesions.

Diaphragm—Negative.

Spleen—Old perisplenitis present. Pulp congested and rather soft. Weight 270 grammes.

Liver—Negative, except for slight congestion. Gall-bladder and ducts negative. Weight 1750 grammes.

Kidneys—Capsules not adherent; pelves rather large. On section both kidneys are congested and somewhat hard. Weight 330 grammes together.

Adrenals—Rather large, congested, medulla thick.

Esophagus—Somewhat dilated, fairly marked keratosis.

Stomach and Intestines—Congestion of stomach. Intestines practically negative, except for cecum, beginning of ascending colon, and lower part of descending colon, all of which show congestion.

Pancreas—Very large and congested. Weight 112 grammes.

Bladder—Negative.

Prostate—Contains few very small calcareous masses.

Spine—A few supra-cartilaginous exostoses.

Brain and Spinal Cord—No gross lesion.

MICROSCOPIC FINDINGS.

The material was fixed in Zenker's fluid, formalin, Marchi's fluid and alcohol, and the sections were stained with hematoxylin and eosin, Van Gieson's stain, Unna's polychrome methylene blue, Mallory's connective-tissue stain, Gram's stain, Sudan III, Nissl's stain and Weigert's myelin stain.

Muscles.—A large number of sections from the psoas, longus colli, deltoid, supinator longus, rectus abdominis and diaphragm were studied and the results were fairly uniform in every muscle. The constant lesion in the voluntary muscles is the presence of smaller or larger areas of cellular infiltration. In all of the muscles examined we were able to find this lesion. In most instances these areas are seen without difficulty; at other times a long search is required and it may be necessary to section many pieces of muscle before a single area can be discovered. The cells in these infiltrations are of small size and uniformly round in shape. Their nuclei are relatively large, usually round or slightly oval in form, and stain intensely with hematoxylin and the nuclear aniline dyes. Morphologically these cells resemble small lymphocytes. No polynuclear leucocytes, plasma cells or eosinophile cells are present in the infiltrated areas, nor can any mitotic cells be seen. In sections where the muscle fibers are cut longitudinally these areas of lymphocytic infiltration are found to be situated between adjacent fibers which are separated slightly as a result. Occasionally the cells may be seen between two or three adjacent fibers. The infiltrations have a tendency, in longitudinal sections, to form long, narrow bands which are easily seen upon examination on account of their intense staining qualities. In the rectus abdominis an area of this kind was found which measures 0.95 mm. in length and 0.078 mm. in

width (see Fig. 1). In transverse sections the appearance is somewhat different. Here the cellular infiltrations are cut in the opposite axis and appear as oval or roundish areas which vary considerably in size. Some may show only 15 or 20 cells while others may present ten or twenty times that number. One of the smaller areas in the psoas muscle measured 0.1 mm. by 0.03 mm., while a larger area measured 0.3 by 0.15 mm. (see Fig. 2). Buzzard has suggested the term "lymphorrhages" for these cellular infiltrations, coining the word to designate that they are composed of lymphocytic cells infiltrating the muscle fibers as do red blood cells in interstitial hemorrhages. This terminology we shall adopt in our paper for the sake of convenience. Most of the transverse sections show from one to three small capillaries in the central part of each lymphorrhage. These capillaries appear quite normal and are often filled with blood (see Fig. 3). In the longitudinal sections it is more difficult to find these small vessels unless serial sections are examined. In the study of the deltoid several longitudinally cut capillaries are seen. It is possible, in some of these vessels, to see the perivascular lymph spaces with ease, on account of the presence of lymphoid cells which completely fill the dilated lymph spaces. In one of the capillaries the endothelium limiting the outer wall of the perivascular lymph space is clearly seen. This condition is identical with that to be described in the thymic tumor. In the other vessels the large number and compact arrangement of the cells obscure these details. The muscle fibers in the immediate vicinity of the lymphorrhages show no degenerative or atrophic changes.

Some of the muscle bundles have a tendency to stain more intensely than others, but this is frequently seen in normal muscles and can not be considered as a pathological process. In transverse sections Cohnheim's fields are well marked in most specimens. The striations are distinctly seen in all sections. In some of the muscles rather prominent bands of connective tissue which stain reddish by Van Gieson's method, may be seen. Some of these bands merge directly into the muscle fibers. At these situations the sarcolemma cells are increased in number and the transverse striations are somewhat indistinct. Some of these connective tissue bands have a more

or less wavy outline and show a few indistinct oval nuclei. They bear a striking resemblance to muscle fibers in shape and size and must be looked upon as a replacement fibrosis secondary to degenerative muscle changes. This lesion is most marked in the deltoid muscle. Otherwise only a normal amount of connective tissue is present. No evidence of fatty degeneration is seen in osmic acid preparations and no pigmentation can be found in any of the muscles. A few fibers are the seat of marked degenerative changes. This is particularly noted in transverse sections of the longus colli muscle. Here the fibers stain distinctly pinkish with Van Gieson's stain, and the muscle nuclei are increased in numbers. A few vacuolated cells with peripherally situated nuclei are also seen. The protoplasm is somewhat granular, quite different from the homogeneous appearance noted in ordinary hyaline degeneration (see Fig. 4). This lesion, as well as that described above as occurring in the deltoid, may be considered as an early degenerative atrophic change.

In the study of the psoas muscle a few small areas are seen which show a different picture. Here the sarcolemma cells are very numerous but the muscle fibers apparently are unchanged. A few cells are also present showing pyknotic nuclear changes. It is difficult to classify properly these cells, but we look upon them as polynuclear leucocytes with degenerating nuclei (see Fig. 5). Throughout the sections the walls of the blood vessels do not show any changes whatever. The medullated nerve fibers are likewise normal, and the sensory nerve-endings ("muscle-spindles") which are present in some of the muscles in large numbers are apparently free from any pathological changes.

Unfortunately the eye-muscles could not be obtained for examination.

Tongue.—After a long search through many sections it was possible to find a few small areas of infiltration in this organ. Apart from their small size these areas do not differ from those found in the skeletal muscles. Otherwise no changes are present, either in the muscular portion, vessels or nerves.

Heart.—It was impossible to discover any lymphorrhages in this organ. A moderate grade of interstitial myocarditis is present,

and a few small masses of hyaline matter may be seen in the connective tissue surrounding some of the larger blood-vessels. The arteries themselves are not affected. The muscle nuclei vary considerably in size and shape and many large irregular forms are seen which represent regenerative changes. The striations are distinct throughout.

Thymus Tumor.—Sections were cut from various parts of the tumor in order to determine its origin and relationship to the thymus gland proper. In only a few places could any normal thymus tissue be found. This, in some instances, was separated from the tumor by layers of connective-tissue, but in other places it was seen to be in direct relationship with the tumor. The thymus tissue has a normal appearance and contains a small number of Hassel's corpuscles, some of which show calcareous changes, while others contain keratohyaline cells and polynuclear leucocytes. In some of the sections the thymus tissue is divided by connective-tissue septa into small lobules. The tumor itself is separated from the surrounding structures by a well-marked connective-tissue capsule and with the low power this is seen to extend into the tumor mass which is thus divided into numerous lobes. The connective-tissue septa widen out in many places, inclosing islands of adipose tissue. Blood vessels of considerable size are present in these bands of connective-tissue, also scattered areas of blood pigment. Occasionally a few small fibers are seen running for a short distance from the septa into the tumor lobules.

Upon examination of the sections with the low power the tumor appears to be dotted with numerous slits, fissures and oval-shaped openings. With a higher power it is seen that these openings are widely dilated lymph channels or lymph spaces lined with endothelium. Some of the larger channels are of considerable length and have parallel walls; others are more or less oval in shape, due to the angle at which the section is cut. The tumor proper is of a very dense cellular structure. The cells are quite large, of an oval, polygonal or irregular shape, and contain large vesicular nuclei with numerous chromatin granules. The cytoplasm is granular and stains lightly with eosin or picric acid. Throughout the sections the cells have a tendency to form concentric bodies of fairly large

size, somewhat resembling those found in psammoma and certain types of endothelioma, but having no direct connection with any blood vessels. As many as twelve, fifteen, or more of these bodies may be counted in a single field. The cells in these concentric bodies are of spindle shape and their nuclei are long, narrow and curved as the result of compression (see Fig. 6). Some of the larger tumor cells contain two or more nuclei. A few cells show vacuolization of the cytoplasm, the nuclei remaining intact. A considerable number of nuclei throughout the sections show distinct nucleoli. None of the cells show any mitotic changes. A delicate stroma may be seen between the cells in those situations where the cells are less densely packed together. In some of the sections small islands of lymphoid cells are seen scattered between the tumor cells. No Hassel's corpuscles are found here, but these areas give the impression of being remnants of thymus tissue. Small masses of hyaline matter are seen here and there in the tumor, some of which are in close proximity to the blood vessels.

The lymph spaces are well filled with large spherical cells which show faint outlines and a coarsely granular cytoplasm. Most of the cells contain a small nucleus of irregular shape which is usually situated at the periphery. Besides these, smaller lymphocytic cells and granular matter are seen filling the spaces. Some of the larger cells show nuclear fragmentation. A few degenerated areas containing cholesterin crystals are seen.

The most interesting feature is the relationship of the tumor cells to the lymph spaces and blood vessels. All of the lymph spaces are lined by endothelial cells, as has been mentioned above. In some fields it appears as though several parallel layers of proliferated endothelium surrounded a considerable portion of the lymph space. These cells always stain more deeply than the tumor cells proper and in some situations form distinct bundles of spindle-shaped cells, merging gradually into the tumor cells. In other places the tumor cells appear to be directly springing from the endothelium of the lymph spaces (see Fig. 7). This is most distinctly seen where the lymph spaces have been cut more or less transversely, and in such instances the cells have a slight tendency to grow radially from their walls.

Blood vessels are very numerous throughout the tumor. These consist principally of newly-formed capillaries, though small arteries with thin walls are also present. Some of the latter show well-marked hyaline degeneration. A zone of lymphocytes surrounds each capillary. This may be seen in transverse as well as in longitudinal sections. In transverse sections these cells appear as a mantle in close relationship to the capillary wall. The anatomical position of these cells can be seen very clearly in longitudinal sections. Here we have a narrow band of cells on either side of the capillary and parallel to it. The cells are seen to be bounded on one side by the endothelium of the capillary and on the other side by a second row of endothelium which must be looked upon as the outer wall of the perivascular lymph space. In other words, these cells are confined to the perivascular lymph spaces. Just beyond these cells are clear spaces due to retraction or shrinkage and bounded by the tumor cells proper (see Fig. 8). The first layer of cells grows at right angles to the vessel walls and this radial arrangement may readily be seen with the low power. They also stain more intensely than those situated at a greater distance from the vessels. There seems to be no doubt that the tumor cells originally sprung from the endothelium of the perivascular lymph spaces, from which they have become detached (see Fig. 9). In a few fields, however, the tumor cells may still be seen in close relationship to the lymph spaces, but as a rule retraction is quite constant. The endothelium of the capillaries is normal throughout and has no bearing on the formation of the tumor.

In this connection it might not be out of place to say a few words regarding the discussion which has arisen concerning the perithelium of bood vessels and its relationship to perivascular tumors. The perithelium of the blood vessels is supposed to represent the endothelium of the perivascular lymph spaces, but in most cases it is difficult or impossible to discover the presence of a second layer of endothelium to form an outer wall of these lymph spaces. In the usual type of perithelial growth with a radial arrangement of the tumor cells these are always closely united to the vessel wall. This is not the case in our tumor, in which a well defined zone containing lymphocytic cells separates tumor cells from vessel wall.

As far as the presence of any outer endothelial boundary is concerned, a study of our case demonstrates such a layer in those sections where the capillaries are cut longitudinally. The fact that a dilatation of all the lymph vessels with marked lymph stasis is present in our case may account for the comparatively easy recognition of the perivascular lymph spaces. It is possible that many of the recorded cases of perithelioma in which the cells appear to arise directly from the adventitia of the blood vessels and where the perivascular lymph spaces are not dilated, belong in this group. Our case demonstrates the fact that tumor cells may encircle and grow radially from a blood vessel and still have no direct attachment to the vessel wall proper, but arise from an outer layer of endothelium bounding the perivascular lymph spaces.

The proper classification of this tumor is somewhat difficult. Inasmuch as the growth is perilymphatic in character, it bears the same relationship to lymphangio-endothelioma as a perithelioma does to haemangio-endothelioma. It is therefore a perilymphatic lymphangio-endothelioma.

Thyroid Gland.—The alveoli are lined by the usual layer of epithelial cells and are well filled with colloid material. The blood vessels are all distended and filled with blood and the picture is that of an acute congestion. Otherwise the sections appear quite normal.

Lungs.—Sections show an intense acute congestion. The alveoli are filled with a finely granular exudate. In some of the alveoli desquamated epithelial cells are seen, many of which are filled with coarse granules of a black or brownish color. A moderate amount of anthracosis is present, most marked in the connective tissue stroma surrounding the larger blood vessels.

Liver.—In this organ a few areas of lymphocytic infiltration are seen. The cells here are in every way identical with those described in the muscles. The areas are rather small and are situated apparently in the capillaries between the liver cells. None of these areas are found in the vicinity of any of the larger blood vessels or ducts. The liver shows a moderate degree of fatty degeneration and chronic congestion, with secondary atrophy and pigmentation of the liver cells.

Spleen.—The capsule shows a moderate grade of perisplenitis.

An intense degree of acute congestion is present. Some of the blood vessels are surrounded by coarse granules of brownish pigment. Hyaline degeneration is noted in the walls of many of the arteries.

Kidneys.—A moderate degree of chronic interstitial nephritis is seen. The epithelium of the tubules shows parenchymatous degeneration. The whole organ is acutely congested and the capillaries of the Malpighian bodies are very prominent.

Adrenals.—In the cortical substance of one adrenal a large lymphorrhage is found. This is situated in close proximity to a small artery. The cells in this area are the same as those described before (see Fig. 10). Otherwise these organs show an acute congestion involving the cortex as well as the medulla.

Pancreas.—This organ is the seat of post-mortem degenerative changes.

The other viscera do not show any pathological changes.

Nervous System.—Sections from the face, arm and leg centers of the motor cortex, and from various levels in the pons, medulla and cord were examined. Special attention was paid to the study of the nuclei of the third, fourth, sixth, ninth, tenth, eleventh and twelfth nerves. No pathological changes were noted in the nerve cells in any of these regions. No degeneration of any tract was found either by the Weigert or Marchi methods. Sections from the hypophysis were also normal.

In the perivascular lymph spaces of several small capillaries lymphocytic infiltrations were found. One of these vessels was situated in the grey matter near the tenth nucleus, the others in the outer part of the pyramidal tract ventral to the olivary body (see Figs. 11 and 12). No lesions were found in any of the larger blood vessels at the base.

REMARKS.

Under this heading only those cases in which an autopsy has been performed or excised muscles examined will be discussed. The first recorded lesion that may be regarded as typical of myasthenia gravis was published by Weigert in 1901 in the pathological study of a case observed clinically by Laquer. In the perimysium, as well as between the fibers of the diaphragm and deltoid, the only muscles

examined, Weigert found small areas of cellular infiltration. These cells resembled for the greater part the small round cells normally present in the thymus. A few epithelioid cells identical with those found in the thymus were also demonstrable. No Hassal's corpuscles were found. There was a similar lesion in the cardiac muscle. Occupying the site of the thymus, but not adherent to the subjacent structures, there was found a soft tumor, 5 cm. by 3 cm. in diameter. Microscopically this tumor showed large hemorrhagic areas in which lay scattered islands of tissue consisting almost exclusively of lymphoid cells but interspersed with a few epithelioid cells. A few Hassal's corpuscles were also present. As the small round cells had apparently invaded the walls of some of the smaller veins and filled their lumina, Weigert concluded that the tumor was a lymphosarcoma, and the cellular infiltrations in the muscles metastases.

Weigert's observations regarding the presence of cellular infiltrations in the skeletal muscles have been confirmed by a number of subsequent observers—Goldflam, Link, Hun, Burr, Buzzard, Steinert, Boldt, Osann, Marburg, Frugoni. Not all of these authors, however, are in accord either as to the nature or the significance of these infiltrations, points that will be discussed subsequently.

It is noteworthy that in eleven cases (including our own) or about 20 per cent. of the cases that have come to autopsy, some abnormality of the thymus has been demonstrable. The earliest case of this group is that of Oppenheim who in 1899 reported a lymphosarcoma of the mediastinum about the size of a small apple occupying the site of the thymus. Weigert's case has already been mentioned. Hun, in 1904, also reported a lymphosarcoma of the thymus, consisting microscopically of islands of epithelioid cells without any tendency to the formation of Hassal's corpuscles. Surrounding these islands were small, round cells resembling lymphocytes. In Case VII of Goldflam's series, a diagnosis of mediastinal tumor, the ultimate cause of death, was made, but no autopsy was obtained. Thymic hypertrophy or persistent thymus has been recorded by Link, Hoedlmoser, Burr and Buzzard (Cases II, IV). Finally, Buzzard's Case V presented a thymus weighing 59.4 gms. and containing in its lower portion a multilocular cyst. Hoffmann

questions the origin of many of the reported cases of primary neoplasms of the thymus, particularly the lymphosarcomata. He believes that the great majority of tumors found in this organ have their origin either in the lymph nodes or other structures in the anterior mediastinum and involve the thymus secondarily, and cites a case where a large lymphosarcoma was found in the anterior mediastinum of a child of 7½ years of age, the thymus remaining intact. Weigert himself drew attention to the absence of infiltration of the tissues surrounding the tumor in his case. This has been noted in both Oppenheim's and Hun's cases as well as in our own. Despite the absence of metastases and of infiltration of the surrounding tissues in our case, we feel justified, in view of the microscopic findings, in concluding that we are here dealing with an actual neoplasm arising primarily from the thymus. Regarding the question of metastases, it is a well established fact that tumors of endothelial origin often remain localized for many years.

Bunge in his text-book on physiology states that the thymus is an organ functioning only during fetal life in warm-blooded animals, a statement supported by the researches of R. Fischl, who extirpated this gland in young dogs and chickens without observing any resulting effect upon growth or development. Even Basch, who preceded Fischl in these experiments, while coming to diametrically opposite conclusions, has not recorded any condition even remotely simulating that of myasthenia gravis. It is not feasible to enter into a discussion of all the physiological experiments conducted with the view of solving the vexed problem of the etiology of this disease. In general it may be said that neither feeding with thymus extract nor its subcutaneous administration has led to any satisfactory conclusion. As Weigert, and subsequently Buzzard, have suggested, it is possible that a certain group of cases presenting the symptom-complex known as myasthenia gravis may ultimately be attributed to an abnormally functionating thymus. That other so-called ductless glands may also play a rôle in the causation of this disease is suggested by a typical case reported by Tilney in which a large adenoma of the glandular portion of the pituitary body was present. The occurrence of Basedow's disease as a complication of myasthenia gravis has been occasionally re-

ported, but there are no grounds to assume a causal connection between these two conditions.

In 1898 Goldflam found in pieces of muscle excised from the deltoid in a case of myasthenia gravis, cellular infiltrations similar to those described by Weigert, but containing no epithelioid cells. At the autopsy performed two years later a lymphosarcoma of the right lung (no microscopic examination) was found. The author concluded that the muscular infiltrations were metastases from the lung tumor. Hun also regards the cellular masses found in the muscles of the chest and upper arm in his case as metastases from the thymic tumor. This view of the metastatic nature of the cells in the muscles is difficult to uphold. Weigert, who first propounded this theory, has himself suggested the possibility that these cells represent a reaction to metabolic processes dependent on a perversion of the thymic function. In Hun's case the passive part played by the lymphoid cells of the thymic tumor as contrasted with the active proliferation of the larger epithelioid cells makes it difficult to understand why the latter should be almost wholly lacking in the muscle metastases. Finally, it may be pointed out that the same condition has obtained in the muscles even in the absence of thymic tumor, as well as in our own case, in which the tumor is an endothelioma incapable of producing lymphoid metastases.

Both lymphoid and endothelioid cells of the types found normally in the thymus have been described by Weigert, Hun, Burr and Steinert. The last author derives the endothelioid cells from the neighboring capillaries. In the remaining cases collections of lymphoid cells alone have been present. Their close connection with capillaries was first pointed out by Link, an observation subsequently confirmed by Hun, Buzzard, Marburg, Frugoni and the authors. A few polynuclear leucocytes were noted by Goldflam and Hun, the latter also describing a few eosinophile cells. A recent hemorrhage was found by Link in one preparation, while Hun records the presence of many red blood cells in the lymphorrhages. The nature of the cellular changes described by Marburg and Frugoni require separate consideration. In addition to lymphocytes among which a few polynuclear leucocytes are scattered, Marburg describes cells resembling the former, but differing in the shape of

their nuclei which are oval and frequently show mitotic division. These he regards as proliferating, young sarcolemma cells. Frugoni believes that many of the cells present in the muscular foci correspond to the "Tochterplasmazellen" of Unna, a few, however, being true plasma cells. In addition, both authors found degenerative changes in the muscle fibers in the neighborhood of the cellular foci. They conclude, therefore, that the latter represent a reaction to a degenerative myositis.

Lymphocytic infiltration in the internal organs were first recorded by Buzzard, who found them in the adrenal in all the cases in his series, in the liver in four cases, and in the kidney, thyroid and peripancreatic fat tissue in one case each. In the liver and adrenal the neighboring cells of the organs had undergone simple degeneration, as the author states, from the pressure exerted by the infiltration. Marburg regards these foci as analogous to the muscular infiltrations; that is, as a reaction to a destructive inflammation affecting the parenchyma cells. The presence of the lymphorrhages in the liver and adrenal we have been able to confirm in our case, but have seen no degenerative changes in the parenchyma cells such as Buzzard describes. Moreover, although extensive muscular changes are present in our case as evidenced by granular degeneration, areas of proliferating sarcolemma cells, and the formation of new connective tissue, these lesions bear no relation to the location of the lymphorrhages. We must therefore conclude with Buzzard that we are here dealing with true lymphocytic infiltrations that are not the result of an inflammatory reaction. In discussing the origin of these cells, Buzzard is inclined to believe that they are derived from the blood current in the capillaries, basing his conclusion upon his findings in the heart in Case III of his series in which the capillaries of this organ were engorged with lymphocytes. In the lymphorrhages in our case these cells very evidently lie in the perivascular lymph spaces, both in the central nervous system and in the muscles. We are inclined to the view that here as well as in the other situations in which they are found, the lymphocytes are derived from the dilated perivascular lymph spaces.

Slight and localized degenerative changes in the muscles have been described by a number of other writers on myasthenia gravis.

Both Link and Goldflam record a few atrophic fibers at the site of the lymphorrhages. Sossedorf and Liefman found an increase in the adipose and connective tissue of the tongue as well as atrophic fibers and evidences of inflammation. Dejerine and Thomas found granular degeneration of the muscle fibers of the tongue and larynx but lay no stress upon its presence. Raymond and Alquier report homogeneous degeneration of the sarcoplasm with fragmentation. Fatty degeneration has been recorded by Senator (a doubtful case), by Marburg as well as by Frugoni in preparations fixed in osmic acid, and by Steinert in fresh preparations. By the last three authors the fat droplets are described as occurring in longitudinal rows simulating the normal striations. As mentioned above we have been unable to confirm this lesion in our case, although numerous sections were studied with this point in view. Tilney found swelling of the muscular fibers, indistinct striations and proliferation of sarcolemma cells. In Buzzard's series of five cases, the fibers of the skeletal and ocular muscles were for the most part normal. Here and there, however, were fibers with rounded contour and diminished affinity for acid stains. Only in Case V, a rapidly fatal case, was the degeneration in the fibers adjacent to the lymphorrhages, more advanced. Here hyaline and granular degeneration were quite marked, a few fibers showing vacuolization and proliferation of the sarcolemma cells. A few lymphocytes infiltrated individual muscle fibers. Similar changes, also in a relatively small number of fibers, were present in the case reported by Frugoni. Muscular atrophy has been reported in only a few cases clinically, most often in the tongue. Histologically, we have been able to find no instance of extensive atrophy recorded although Buzzard considers that the changes found in the muscles in his series of cases may represent a beginning atrophy of the fibers. In our case the muscular lesions are mostly degenerative in nature, but atrophic fibers are present in most of the sections made from the deltoid and longus colli muscles. The situation of these atrophic changes would correspond to the atrophy of the deltoid noted in the clinical examination.

Although numerous minor changes in the nervous system, as well as a few anomalies, have been described by different investi-

gators, no constant pathological lesion to which the recognized symptom-complex can be attributed has as yet been demonstrated. The lesions of the central nervous system recorded in our collected series may be thus summarized:

I. Anomalies of brain and spinal cord.
   - (*a*) Reduplication of central canal in dorsal and lumbar segments of cord. In the latter situation three canals all lined by epithelium pursue a short course (Senator I).
   - (*b*) Analogous condition in the lower and middle thirds of the aqueduct of Sylvius (Oppenheim II).
   - (*c*) Median dorsal fissure in cord from third to fifth lumbar segments. Pons, medulla and cerebellum smaller than normal (Burr and McCarthy).
   - (*d*) Cellular proliferation near central canal (Link).
   - (*e*) Obliteration of central canal by primitive ependymal cells (Frugoni).

II. Anomalies of cranial nerves.
   - (*a*) Abnormal thinness of trunks (Eisenlohr, hypoglossus, vagus and accessorius roots) (Liefman, both abducens).
   - (*b*) Primitive fibers without degeneration (Oppenheim, II, in both hypoglossi) (Eisenlohr, in hypoglossus, accessorius, vagus, facial).
   - (*c*) Slight discoloration of facial nerves and of the anterior roots of some of the spinal nerves (Jolly).

III. Degeneration of fibers of one or more cranial nerves. Sossedorf, Widal-Marinesco, Dejerine and Thomas, Burr and McCarthy, Mayer, Batten and Fletcher, Fajersztayn.

IV. Changes in cells of nuceli in medulla and pons (chromatolysis of Marinesco).

V. Leptomeningitis (Long and Wiki, Osann).

VI. Cyst in anterior lobe of left hemisphere following old hemorrhage (Berkeley).

VII. Homogeneous masses (probably coagulated lymph, Marburg) in the cord, medulla, pons and roots of facial nerves. Where Marchi's fixing fluid has been employed these masses stain black.

VIII. Recent hemorrhages, subpial or in or about nuclei of brain stem (many authors).

Scattered hemorrhages have been the most frequently observed lesion in the nervous system, but these have always been designated as recent and attributed to the suffocative symptoms immediately preceding death. The utter lack of uniformity in the lesions thus tabulated is in itself sufficient to demonstrate that the clinical picture of myasthenia gravis cannot be dependent upon them. As Marburg correctly states, all of the changes recorded in the nervous system have been found either in pathological conditions bearing no relation to myasthenia gravis, or even in apparently normal cases.

Infiltrations in the nervous system have heretofore been recorded only by Buzzard who found small collections of lymphocytic cells in a number of the posterior root ganglia. While we have been able to demonstrate the presence of several lymphorrhages in the medulla, their situation and scarcity seem to indicate that they have no bearing on the bulbar phenomena manifested clinically. Their pathological significance will be discussed subsequently.

In none of the recorded cases have any changes been found in the peripheral nerves. Unfortunately these were not preserved for examination in our case.

PATHOGENESIS.

The pathological and physiological data at our command are too inconclusive to warrant the formulation of a pathogenetic theory of myasthenia gravis. Many such theories, some of which have already been discussed, have been advanced. Among earlier writers, Oppenheim classifies the disease as a neurosis, possibly based upon a congenital anlage. In support of this view he cites that group of cases in which somatic anomalies or anomalies of the nervous system have been present. Etiological significance has also been ascribed to the toxemia arising either from tumors of thymic or other origin, from coprostasis, from pregnancy or from various antecedent infectious disease. Among more recent writers particular stress has been laid upon disturbances of metabolism. As the result of his investigations along these lines Kaufman con-

cludes that the cause of myasthenia is to be found in insufficiency of the metabolic functions of the liver. Only three cases of our series, however, contain a definite statement as to a diseased condition of this organ (cirrhosis, Raymond and Alquier, Boldt; Banti's disease, Mohr). Sitsen records a case in which the preponderance of polynuclear leucocytes in the capillaries of the muscles and internal organs points to leukemia. General lymphatic hyperplasia was present in this case as well as in that reported by Hoedlmoser. Sitsen includes in this group all those cases in which thymic hypertrophy has been recorded, and suggests that the causative toxic agent of myasthenia may arise in the lymphatic system.

It would consume too much space to discuss in detail all the theories that have been propounded. In general, it may be said that a review of the literature demonstrates the notable inconstancy in the pathaological, anatomical or anamnestic findings upon which they have been founded. Our case does not enable us to throw any additional light upon the question of pathogenesis, but our findings seem to warrant the following conclusions:

1. Neoplasms of thymic origin have been noted too frequently in myasthenia gravis to be ignored as a possible etiological factor in a certain proportion of the cases. In our case an unusual type of tumor, hitherto undescribed in this disease, is present.

2. While definite proof is still lacking, it seems most probable that the disease is the manifestation of a toxemia of indeterminate origin.

3. The action of the toxic agent is not confined to the muscular system, but the organism is generally effected as evidenced by the widespread presence of lymphocytic infiltrations throughout the body.

4. Although no degenerative changes are demonstrable in either brain or cord, the occurrence of lymphocytic infiltrations in the medulla, observed for the first time in our case, indicates the involvement of the central nervous system in the general toxemia.

5. The changes in the muscle fibers are purely degenerative, the result of the toxemia, and not dependent upon a primary myositis. These degenerative lesions bear no relation to the site of the lymphocytic infiltrations.

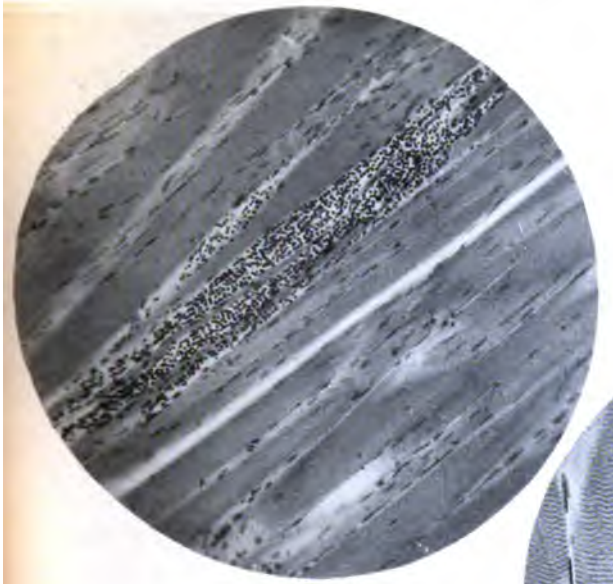

FIG. 1.

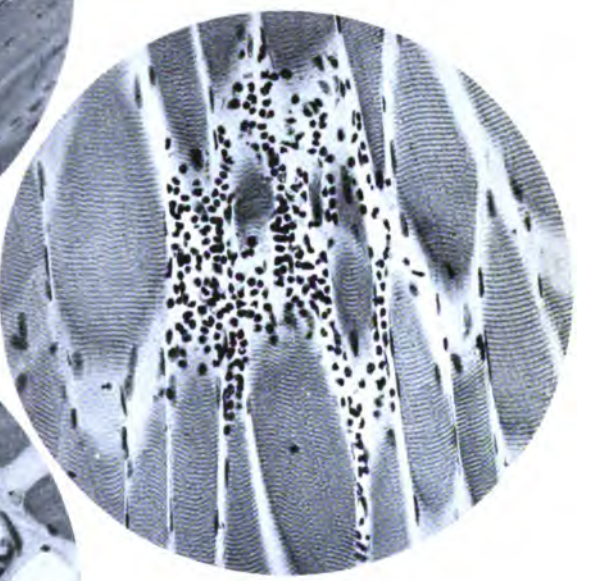

FIG. 2.

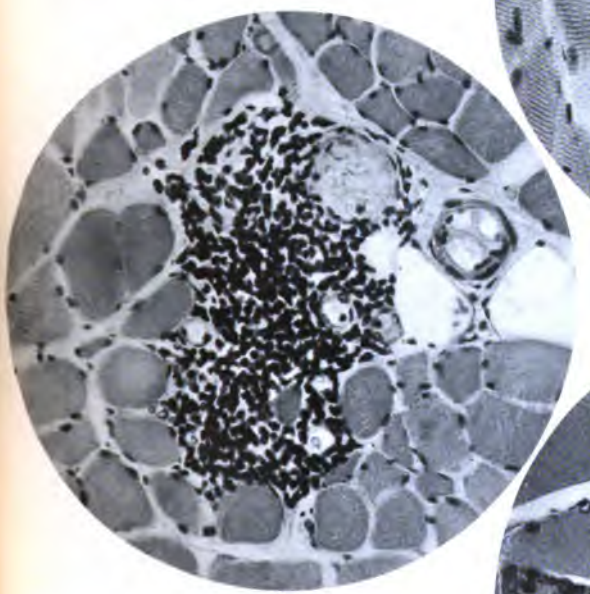

FIG. 3.

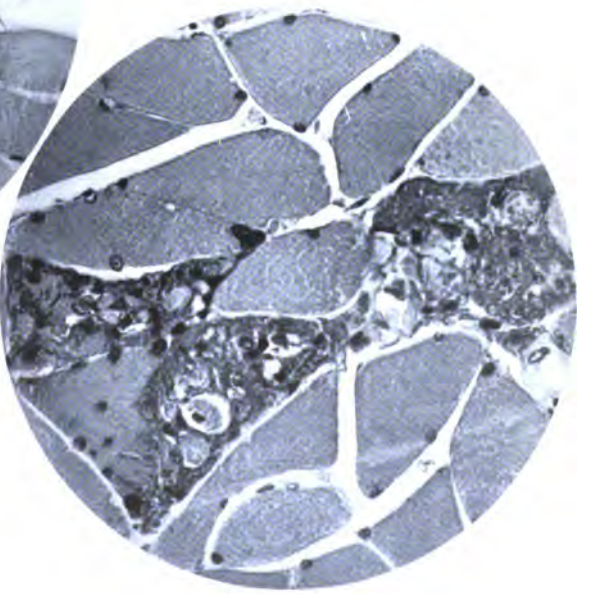

FIG. 4.

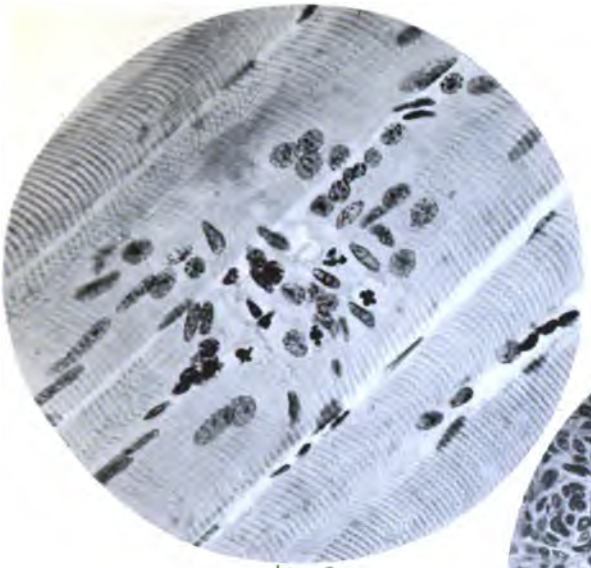

FIG. 5.

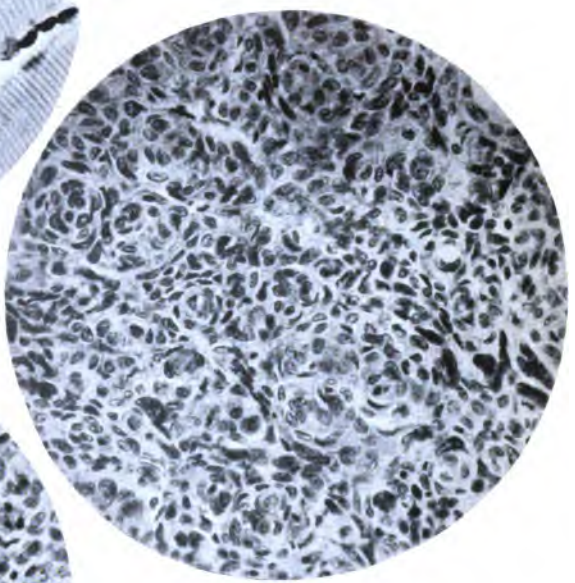

FIG. 6.

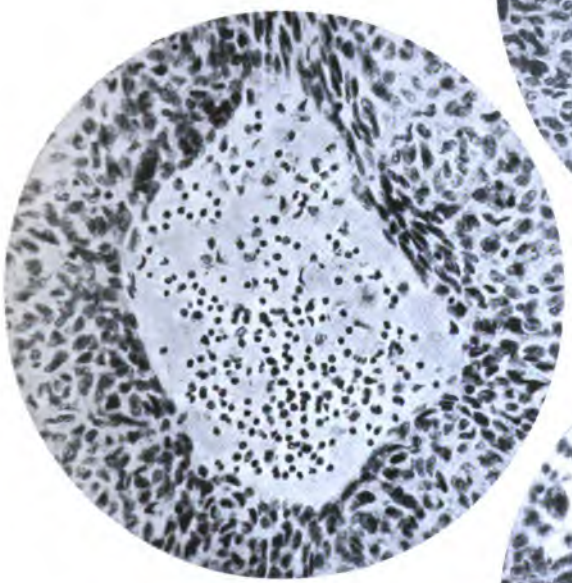

FIG. 7.

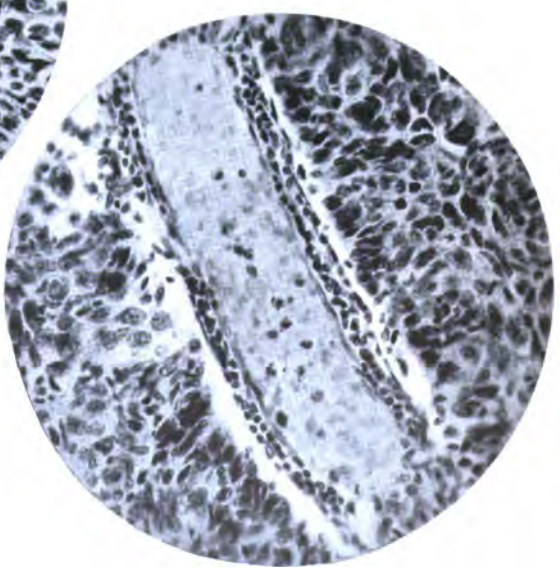

FIG. 8.

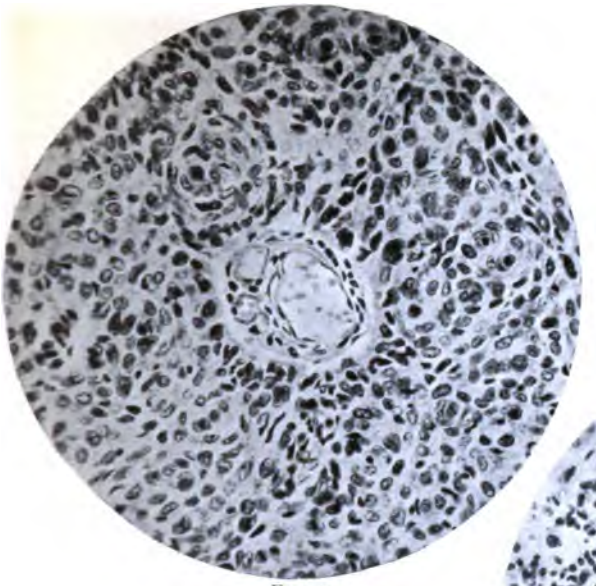

FIG. 9.

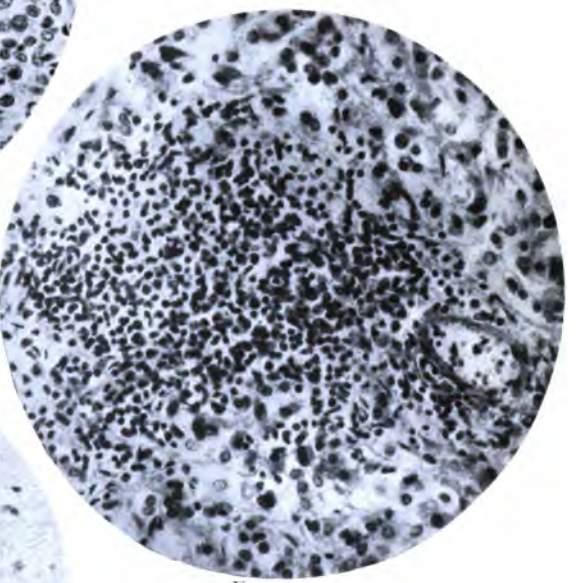

FIG. 10.

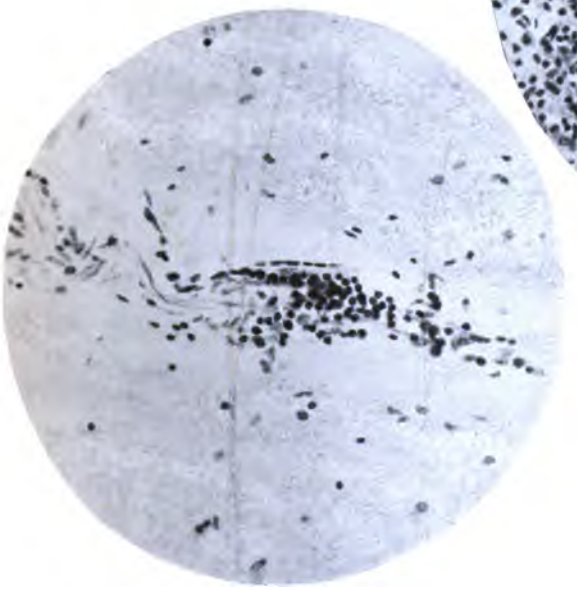

FIG. 11.

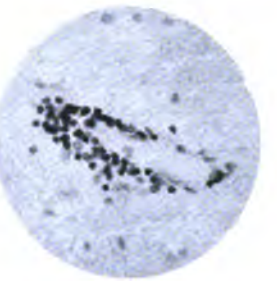

FIG. 12.

6. A study of our specimens seems to show that the lymphoid cells, wherever present in the tissues, are derived from perivascular lymph spaces.

In conclusion we desire to express our thanks to Dr. I. Strauss for his valuable assistance in the study of the nervous system.

Explanation of Plates XXI–XXIII

Fig. 1. A long band of lymphocytic infiltration in the rectus muscle. × 125.

Fig. 2. Section of the psoas muscle showing the usual type of lymphocytic infiltration. × 250.

Fig. 3. A cross section of the deltoid muscle with several small capillaries surrounded by a dense infiltration. × 250.

Fig. 4. A cross section of the longus colli muscle showing granular degeneration, vacuolated cells and proliferation of muscle nuclei. × 500.

Fig. 5. From a section of the psoas muscle, showing proliferation of the sarcolemma cells, and five cells with pyknotic nuclear changes. × 500.

Fig. 6. From a section of the thymus tumor, showing the general type of growth and numerous concentric bodies. × 250.

Fig. 7. A section of the thymus tumor showing a dilated lymph sinus filled with large, faintly-staining cells and small lymphocytic cells. The tumor cells are seen springing from the endothelium of the sinus. × 250.

Fig. 8. Longitudinal section of a capillary in the thymus tumor. The capillary wall is outlined by a dense collection of lymphocytes situated in the perivascular lymph space and bounded on either side by endothelium. The tumor cells have slightly shrunken away from the outer layer of endothelium. × 250.

Fig. 9. Transverse section of capillaries in the thymus tumor. Just outside of the vascular endothelium is a mantle of lymphocytic cells in the perivascular lymph space. The tumor cells have retracted and show a radial arrangement. × 250.

Fig. 10. A section of the adrenal showing a large area of lymphocytic infiltration near a small artery. × 250.

Fig. 11. Longitudinal section of a capillary in the gray matter near the tenth nucleus, showing lymphocytic infiltration in the perivascular lymph space. × 250.

Fig. 12. Transverse sections of two capillaries in the outer part of the pyramidal tract ventral to the olivary body, showing the same lesion as Fig. 11.

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Note.—Since the completion of the above paper, Chvostek (*Wiener klinische Wochenschr.,* 1908, xxi, 37) has advanced the theory that myasthenia gravis is dependent upon hypersecretion of the parathyroid glands. He bases his hypothesis upon the purely theoretical grounds that both tetany and myasthenia affect the neuromuscular system but are diametrically opposed in their clinical manifestations, and, since tetany arises from hyposecretion of the parathyroid glands, myasthenia must arise from a hypersecretion of these bodies. This theory cannot be accepted without further investigation along these lines.

THE EFFECT OF PILOCARPINE ON THE OUTPUT OF LYMPHOCYTES THROUGH THE THORACIC DUCT.*

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The lymphocytosis induced in the blood by pilocarpine is a phenomenon which has been turned to the use of many theories but has had, of itself, little study. Horbaczewski (1891) (1) discovered that the drug increases the white cells. The finding helped him to work out his idea of the dependence of uric acid excretion on leukocyte destruction. Ruzicka (2) obtained a profound leukocytosis in rabbits by the intravenous injection of large doses of pilocarpine. He could not account for the rapid occurrence of this result, but thought proliferation in the hæmatopoietic tissues responsible for its continuance. Waldstein (1893) (3), assuming that an increase of the mononuclear elements in the blood would influence favorably the course of some infectious diseases, gave small amounts of pilocarpine at intervals of several days, and obtained as result a large, absolute lymphocytosis. Recently Lefmann (4) and Gasis (5) have repeated Waldstein's experiments with rabbits, in demonstration of an effect of the Roentgen rays to bring about quick disappearance of the lymphocytosis.

The evidence seems good that pilocarpine given in small doses over a considerable period of time produces a lymphocytosis absolute in type. The immediate effect of the drug is to cause (in rabbits) a general increase of the white cells, involving especially the lymphocytes. This result is often cited as an example of possible chemiotactic influence on the lymphocyte, but Harvey (6) has produced evidence to prove it due to contraction of the smooth muscle of the lymph-glands and spleen. Unfortunately his work

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is based on counts of but one hundred cells each from blood smears. There have been no other investigations on the cause of the quick change in the blood picture.

A method adopted by the author (7) for study of the cell-output through the thoracic duct has given him opportunity to note in dogs the immediate effect of pilocarpine on this source of the lymphocytes. In brief, considerable quantities of lymph (3 cubic centimeters to each specimen) are collected from the thoracic duct in specially graduated tubes containing 3 cubic centimeters of 4 per cent. sodium citrate solution in 0.8 per cent. salt solution, and the white cells per cubic millimeter estimated from the mixture after it has been thoroughly agitated. This estimation is accomplished with melangeur and counting-chamber in the ordinary manner. The mixture of lymph and sodium citrate solution is not diluted, but the addition to it of a trace of a saturated aqueous solution of methyl violet (5B) facilitates the counting. The accuracy of this method of cell-enumeration, and the slight variation in the number of cells per cubic millimeter of lymph voided from the thoracic duct during the first two hours after the establishment of a lymph-fistula in an animal quiet under morphia and chloroform, have been shown in the paper cited. The output of cells as a whole becomes gradually less during this period, as the amount of lymph voided gradually lessens.

The dogs used were given, one hour before operation, 0.5 centigram of morphia sulphate per kilo of body weight, and later chloroform to complete the anaesthesia. A cannula was introduced directly into the thoracic duct; the lymph allowed to flow free; and, after the collection of one or more specimens, a small dose of pilocarpine nitrate, dissolved in a few minims of salt solution, was injected intravenously and further collection of lymph made. In every instance the action of the drug was evident within the minute through increase in the saliva.

Since figures dealing with the effect of pilocarpine on the blood of the dog are lacking, preliminary counts were obtained on animals treated as above outlined, except that no lymph-fistula was produced, and the only operation was that necessary to give access to the left, external jugular vein, into which the injections were made.

Throughout the term of observation the animals were kept quiet under morphia and chloroform. In each instance food was withheld during the twenty-four hours previous to operation. Blood for the counts was obtained by nicking with scissors small superficial veins on the abdomen. The cover-glass preparations were stained with Wright's stain, and for each differential count (of 500–600 cells) at least two smears were used. In the table that follows the number both of large and of small mononuclear cells is given.

Experiment I.—Bull-dog, female; wt. 16.5 kilo.

Time.	Procedure.	Total wbc. per cmm.	Small mns. per cmm.	Large mns. per cmm.	Total mns. per cmm.	Rbc. per cmm.
A. M. 9:50	Operation.					
10:15	First count.	18,600	986	484	1,490	6,672,000
10:45	Second count.	18,200	746	528	1,274	
10:50	Pil. nit. 20 mg. intravenously.					
11:22	Third count.	24,900	1,619	896	2,515	6,960,000

Experiment II.—Fox-terrier, male; wt. 9 kilo.

Time.	Procedure.	Total wbc. per cmm.	Small mns. per cmm.	Large mns. per cmm.	Total mns. per cmm.
A. M. 9:40	Operation.				
9:48	First count.	6,400	749	179	928
10:23	Second count.	9,720	846	136	982
10:26	Pil. nit. 10 mg. intravenously.				
11:13	Third count.	15,200	1,870	274	2,144

Experiment III.—Pointer, male; wt. 22 kilo.

Time.	Procedure.	Total wbc. per cmm.	Small mns. per cmm.	Large mns. per cmm.	Total mns. per cmm.
A. M. 9:30	Operation.				
10:30	First count.	13,000	959	429	1,388
10:35	Pil. nit. 10 mg. intravenously.				
11:05	Second count.	19,200	2,170	499	2,669
11:30	Third count.	22,800	2,280	684	2,964

Generalization from these few observations is hardly warranted; yet there seems ground to suppose that pilocarpine, intravenously given, produces a prompt, moderate increase in the mononuclear cells, especially the lymphocytes, of the blood of the dog. Certainly no such extreme lymphocytosis takes place as Harvey noted

in the rabbit. A leukocytosis affecting the polymorphonuclear elements also made its appearance, but one cannot rule out the operation itself as sole cause of this. On the other hand, an absolute increase in the mononuclear cells, such as occurs here, is not a characteristic of the well-known leukocytosis due to operation. The dependence of the lymphocytosis on pilocarpine injection is indicated, furthermore, by those two instances in which repeated counts were made after the animal had been operated upon, but before the administration of the drug. In these counts the number of lymphocytes was found to be practically unvarying.

The direct cell-output by way of the lymph was now studied according to the method already described. It may be remarked in passing that lymphocytes are alone present in the dog's lymph in large number. (Delamere (8), Biedl and v. Decastello (9).)

Experiment IV.—Mongrel, male; wt. 8.2 kilo. Food was withheld from the animal for 18 hours previous to experiment. The duct was opened, and the lymph allowed to flow for 15 minutes before the first specimen was collected. It was pinkish, slightly opalescent, and at no time clotted in the cannula. When one specimen had been obtained 12 milligrams of pilocarpine nitrate were injected into the left, external jugular vein. The respirations became dyspnœic for about one minute, after which they resumed their previous rhythm. The lymph flowed faster and was nearly colorless. Five specimens of it were collected, then a second injection of pilocarpine (11 milligrams) given, and two more specimens obtained. The cell-counts were made from the tubes in the order of their collection and 2 to 3 hours following it. (See Chart 1.)

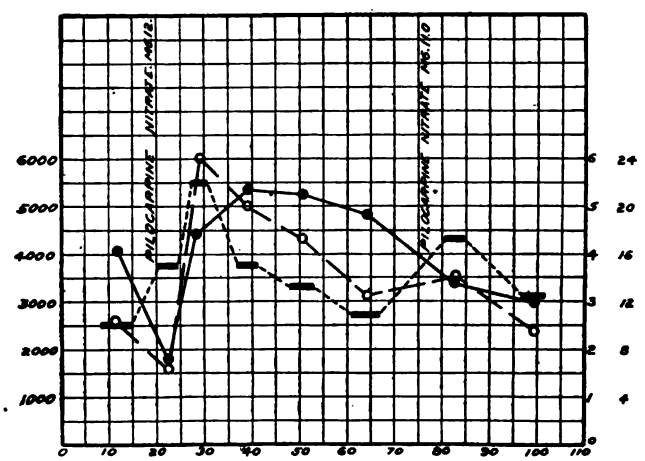

CHART I.

An autopsy proved the animal to have been sound.

Experiment V.—Mongrel hound, male; wt. 9 kilo. Food was withheld for 26 hours previous to the experiment. The lymph was opalescent. A cannula was introduced into the duct after it had been ligated 5 minutes, and 26 minutes prior to the collection of the first tube. Slight clotting in the cannula necessitated twice in the two hours the use of a hooked wire to clean the bore. In one instance the flow was momentarily interfered with. This happened in an interval when lymph was not collected, and with the return of the flow 15 minutes were allowed to pass before another specimen was taken. The injection of the 5 milligrams of pilocarpine into the left, external jugular vein did not cause dyspnœa or movement. Two tubes of lymph were collected during the hour following. Cell-counts were made in the order of collection of specimens and 1½ hours after each had been obtained. (See Chart 2.)

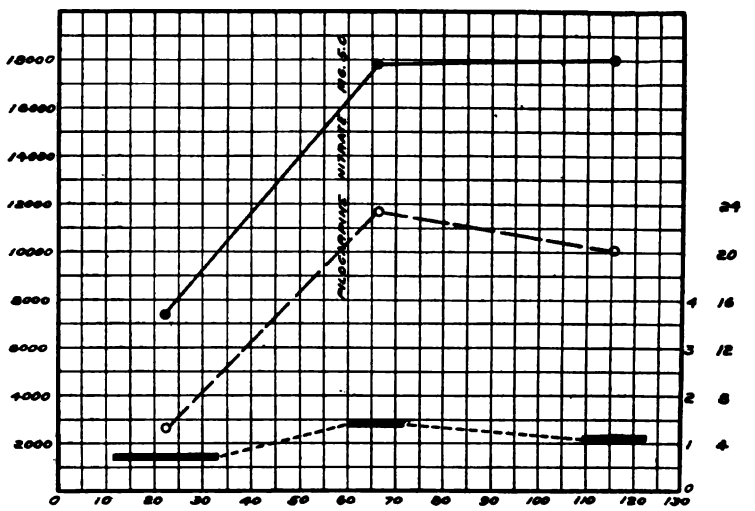

CHART 2.

The slow flow of lymph suggested the presence of an accessory thoracic duct. Accordingly, the duct proper was ligated before the animal was killed, and, with the aid of the natural injection, a search made for branches to the right side of the neck. None connected with the thoracic duct or receptaculum were found. The duct contained no clot and was patent.

Autopsy showed the animal to have been sound.

Experiment VI.—Male, collie; wt. 18.5 kilo. Food was withheld for 26 hours before operation. The thoracic duct was opened after 5 minutes ligation, and 20 minutes allowed to elapse before the collection of lymph was begun. Once in this interval the dog was partly roused by tweaking the skin, that the lymph-system might be flushed, through the quickened lymph-flow incident to struggle, of possible cell-accumulation in its channels. In the quiet following two tubes of lymph were taken, and after this 10 milligrams of pilocarpine nitrate

injected into the left, external jugular vein. The lymph, previously opalescent, became for 15 minutes quite milky, so that a fat ring developed in it on standing. The animal remained quiet and the character of its respirations did not change. Four more tubes were collected, then atropine sulphate, 0.6 milligrams, dissolved in a few minims of normal salt solution, was injected into the left subclavian vein, and two more tubes taken. The drug caused almost immediate cessation of bowel-noises, the flow of salivary secretion stopped, and the lymph slackened markedly in flow, and became clear and slightly blood-tinged. The subsequent injection of 15 milligrams of pilocarpine nitrate did not quicken its flow. There was no clotting in the cannula at any time. The tubes were counted in the order of their collection, and 1½ to 3 hours after it. (See Chart 3.)

At autopsy the animal was found to have been sound. The thoracic duct showed no branch leading to the right side of the neck.

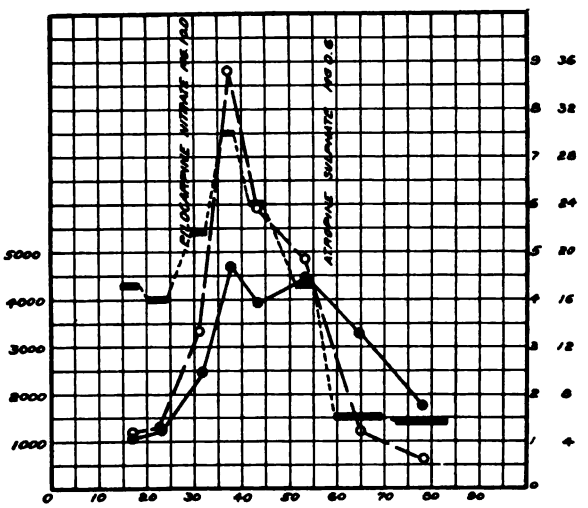

CHART 3.

The height above the base-line of the curve representing amount of lymph-flow indicates the number of cubic centimeters voided through the thoracic duct in a given time; and the black rectangles show the period required to collect the three cubic centimeters of lymph in each specimen. Thus the curve depicts in two ways the rapidity of lymph-flow.

The results of a first injection of pilocarpine in these three experiments are very similar. A well-defined increase in the number of white cells per cubic millimeter of lymph ("cell-concentration") is brought about, as also an increase in the total output of cells. The effect is fairly sustained, lasting one half to one hour. A quickening of the lymph-flow is also seen, but it is not so enduring.

One questions immediately whether the large cell-output is not a corollary to quickened lymph-flow. But comparison shows the two phenomena to lie in only a rough time-relation. Furthermore, as the following experiment demonstrates, pilocarpine will produce a profound increase of cell-output in a lymph-stream that varies little in flow. The effect of the drug is not always that of a lymphagogue (Heidenhain, Tschirwinsky (10), Spiro (11)).

Experiment VII.—Coach-dog, male; wt. 9.2 kilo. The animal was fed with lean beef 3½ hours before operation. The duct was opened after 8 minutes ligation, and the lymph allowed to run for 19 minutes before the first specimen was collected. It was milky, and showed no tendency at any time to clot in the cannula. Two tubes were taken, and then 6 milligrams of pilocarpine nitrate injected into the left, external jugular vein. The animal continued quiet, and the breathing did not change in rhythm, yet during the next 20 minutes the content in fat of the chyle was much increased, as shown by a comparison of the fat-rings that formed in the tubes after they had stood for some hours. The fluid that ran later resembled thin chalk and water. Six specimens were collected, a second injection (5 milligrams) given, and three more tubes obtained. Toward the close of the experiment the breathing was slightly labored, and rhonchi could be heard. The tubes were counted in the order of their collection, and 2½ to 4 hours after it. (See Chart 4.)

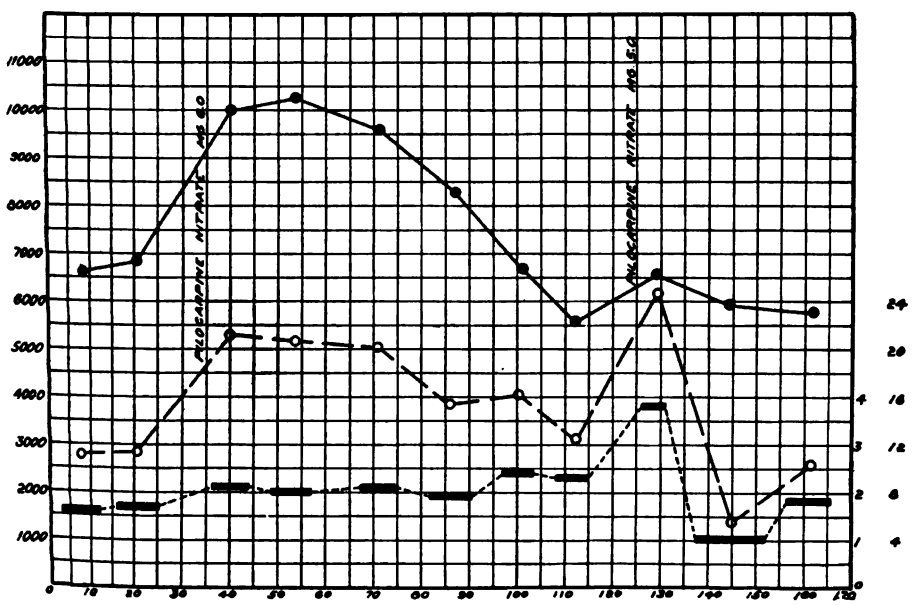

CHART 4.

The autopsy showed the animal to have been sound.

In this instance the effect on the cells of the first injection of pilocarpine was outspoken, despite the nearly constant lymph-flow. The cell-increase was prompt here, as in Experiments IV, V and VI. In all it took place in the ten minutes immediately after the injection.

The actual increase in cell-output is of interest as an indication of the extent to which contributions through the thoracic duct may be responsible for the pilocarpine lymphocytosis. The animals used for the work just presented moved voluntarily, or were induced to struggle, shortly before the experiment proper, to rule out that accumulation in the lymph-system of mature cells, which has been observed to occur in the quiet animal (Goodall and Paton (12), Rous). But the rush into the circulation of accumulated cells when pilocarpine acts on an animal previously quiet is to be reckoned with as an effect of the drug. The next experiment illustrates this.

Experiment VIII.—Collie, male; wt. 19 kilo. No food was given for 50 hours prior to the experiment. The animal was quiet for 1 hour before the collection of the first lymph-specimen which was taken 5 minutes after the opening of the thoracic duct. This had been 1 minute ligated. The slightly opalescent lymph showed no tendency to clot in the cannula. After two tubes of it had been obtained 10 milligrams of pilocarpine nitrate were injected into the left, external jugular vein. The breathing immediately became somewhat dyspnœic and remained so. Five tubes of lymph were taken, then a second injection of 10 milligrams given, and four more tubes obtained. Cell-estimations were made on the specimens in the order of their collection, and 2½ to 3½ hours after it. (See Chart 5.)

At autopsy a large mass of tape-worms was found in the small intestine. Otherwise the animal had been sound. The thoracic duct gave off no branch to the right side of the neck.

If one neglect the action of pilocarpine in changing the fluid content of the blood, a calculation is possible of the absolute increase in lymphocytes per cubic millimeter of blood which would have been caused by such an addition of cells as this.[2] The increased flow of lymph induced by the drug can hardly be supposed to act as a real diluent, since new lymph-production and active secretion from the salivary and other glands tend to drain the blood of fluid. On the basis that the dog, which weighed 19 kilo, had 7.7 per cent. of its

[2] After the administration of pilocarpine the bulk of white cells in the lymph is still one of lymphocytes.

weight in blood of a specific gravity of 1.055, one may assume 1,385 cubic centimeters as the total volume of blood. During the forty minutes following pilocarpine injection an average of 111,-400,000 more white cells were given off through the thoracic duct in each five minutes than during the same period of quiet,—or a total excess over the normal outpouring of 891,000,000 in the

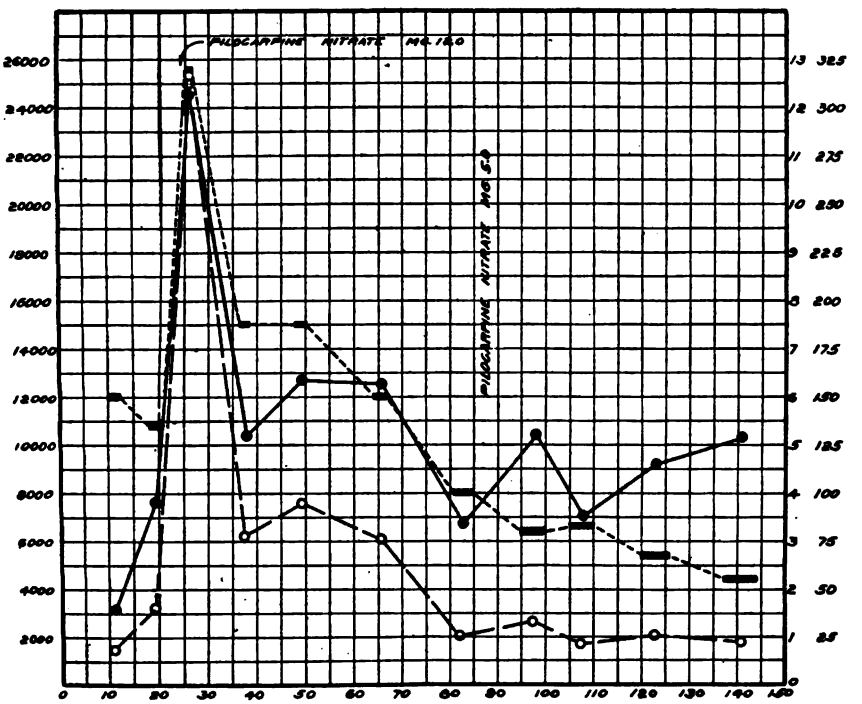

CHART 5.

forty minutes, a number sufficient to have furnished each cubic millimeter of blood with 643 lymphocytes over the normal supply.

The absolute increase in output of lymphocytes through the thoracic duct was much smaller in the other animals: enough in Experiment IV to have furnished 100 lymphocytes to each cubic millimeter of the dog's blood; in Experiment V sufficient for 382 per cubic millimeter; in Experiment VI enough in the short period of observation to give 84 extra cells per cubic millimeter; in Experiment VII enough for 146 extra cells per cubic millimeter. But

in Experiment VIII alone had the animal lain quiet as in Experiments I, II and III. In these three the increase in the circulating lymphocytes that took place in the first thirty to fifty minutes after pilocarpine injection was close to 1,000 per cubic millimeter. An output such as that obtained, despite the unfavorable condition of lymph-fistula, in Experiment VIII, would account for more than half of this lymphocytosis.

The effect of pilocarpine on cell-output through the thoracic duct, as here studied, is probably dependent on several factors:

1. *Increase in Lymph-Flow.*—Others have proved that pilocarpine often, though not always, acts as a lymphagogue. In a previous paper it has been shown that increase in lymph-flow alone,—the factor invoked by Ehrlich for the production of quickly appearing lymphocytosis,—exerts indeed a considerable influence to increase cell-output through the thoracic duct. When a condition of bodily quiet has allowed accumulation of cells in the lymph-system the number flushed out with a quickened lymph-stream may be large, as in Experiment VIII. Yet that the increased cell-output is but secondarily dependent on this factor has been made clear.

2. *Dyspnœic Breathing.*—This is frequently induced by pilocarpine (Cushny (13)). In one only of the five experiments was it marked, though in a second it was briefly present. By its pumping action on the great lymph-channels of the trunk it tends to keep their contents in motion (Starling (14)), and would hinder in this way cell-accumulation.

3. *Contraction of Smooth Muscle.*—Pilocarpine contracts the lumen of the large lymph-vessels (Heinz (15)). Obviously a result of this narrowing is a very brief increase in the amount of lymph voided through the thoracic duct, and, as this is cell-containing, the total cell-output would also be briefly increased.

Harvey believes that contraction of the smooth muscle of lymph-glands and spleen is entirely responsible for the lymphocytosis he observed to follow pilocarpine injections in rabbits. He bases his conclusion principally on the fact that atropine prevents the occurrence of this lymphocytosis, whereas it does not hinder the occurrence of that which he found barium chloride to produce.

Whatever may be said of the effect of barium chloride on the

blood, it is certain that Harvey's atropine-pilocarpine experiments admit of a second interpretation as regards the process taking place in the lymph-system. For it is well-known (Spiro, Tschirwinsky) that atropine slows the lymph-flow strikingly, even though comparatively large doses of pilocarpine be given. Its action is in this way antagonistic to the increase of cell-output through the thoracic duct; for no matter if some force liberated cells from the lymph-glands, the stagnant current would prove but a poor medium for their transport. In illustration the effect of atropine in Experiment VI may be pointed out. Given shortly after pilocarpine, it immediately reduced lymph-flow and cell-output to less than they had been previous to the administration of either drug. The following experiment furnishes a further illustration:

Experiment IX.—Bull-dog, male; wt. 25 kilo. No food was allowed it for 24 hours previous to the experiment. The thoracic duct was opened after 3 minutes ligation, and the lymph ran free during 17 minutes before collection was begun. It was clear and at no time clotted in the cannula. Two specimens were taken to establish the facts of output, then 1.2 milligrams of atropine sulphate in a few minims of salt solution were injected into the left,

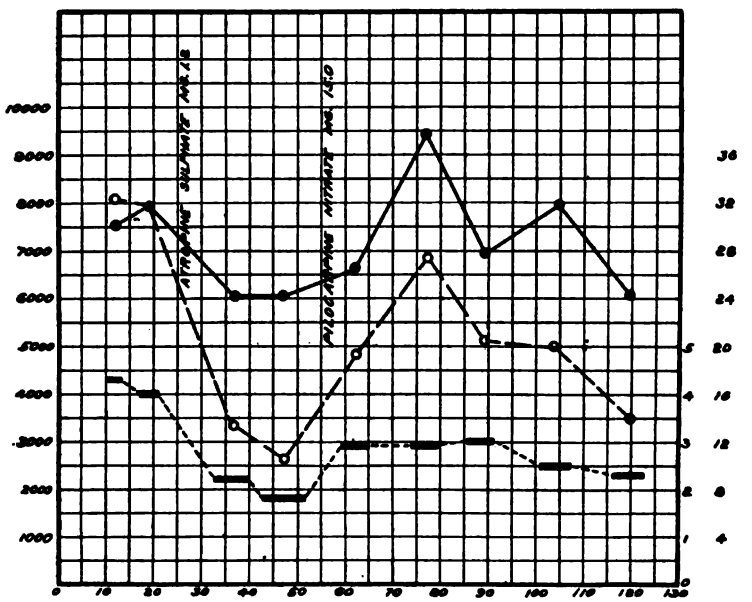

CHART 6.

external jugular vein, and, with decreased lymph-flow manifest, two more specimens obtained. The animal remained quiet. Fifteen milligrams of pilocarpine nitrate were now injected into the left, external jugular vein, causing a momentary flow of saliva, a few dyspnœic movements of the chest, and twitching of the limbs, all of which ceased within the minute. No other changes in the animal's condition were noted. Five more tubes of lymph were obtained. Cell-counts were made in the order of tube-collection, and from 2 to 3 hours after it. (See Chart 6.)

Autopsy showed the animal to have been healthy. There were many tapeworms in the large intestine.

In this instance atropine reduced the lymph-output to one third its previous quantity, and cell-output by way of the lymph to much less than half. Pilocarpine raised the lymph- and cell-output again, but not to their rate previous to atropinization. It is impossible to say that the changes in cell-output are not wholly dependent on those of lymph-flow. Similarly, the profound fall in cell-output brought about in Experiment VI by atropine may be due to nothing else than lessened lymph-flow. To seek a further factor is unnecessary.

Yet some action of pilocarpine to further cell-output, other than those of increased lymph-flow and dyspnœic breathing, is certainly present. Stimulation of the lymph-glands to productive activity cannot be responsible, since the increase in cell-output occurs practically at once. Were chemiotaxis a factor, as Gulland (16) believes, a second injection of pilocarpine ought to influence cell-output. But instances (Experiments IV, V, VI) of a second injection show it to have practically no effect. Contraction of smooth muscle must be further considered.

As has been demonstrated, the effect of atropine on the lymphocytosis of pilocarpine is neither for nor against its origin by smooth muscle contraction. That direct pressure (such as this contraction would bring about) may increase the cell-output of the lymph is highly probable, since the pressure exerted by a quickened lymph-flow will increase it. In this connection it is noteworthy that pilocarpine may render briefly chylous a lymph previously opalescent, or may, during a short period, increase the fat in one already milky (Experiments VI and VII). Either the absorption of fat from the intestine is for a brief time aided by the pilocarpine, or a larger proportion of intestinal lymph in the "mixed lymph" of the tho-

racic duct causes the latter to appear more chylous. The active movements of the intestine brought about by the drug, in association with Heidenhain's observation that much lymph may be squeezed out of the lacteals by direct pressure on a loop of the gut, makes this latter supposition probable; while the fact that the increase in fat of the "mixed lymph" appears abruptly and is transient speaks against the idea of an increase in absorption. Now, as is well known, the intestines and mesentery form the area of lymph-supply richest in lymph nodes and lymphoid tissue; and pressure changes in this area, taking place through contraction of the smooth muscle, may well be supposed to increase the output of white cells through the thoracic duct.

SUMMARY.

The intravenous injection of pilocarpine nitrate causes in the dog a rapid and considerable increase in the output of lymphocytes through the thoracic duct. The corresponding lymphocytosis induced by the drug in the blood of this animal is not profound, and increased cell-output with the lymph will explain a large part if not all of it.

Quickened lymph-flow and dyspnœic breathing are accessory in the production of the large cell-output with the lymph, but it is mainly dependent on some undetermined element. The evidence points to the mechanical nature of this element. It is probably to be sought in direct pressure from contraction of smooth muscle, as suggested by Harvey, but his observation that atropine prevents the appearance of a lymphocytosis after pilocarpine cannot be quoted in proof because atropine much slows the lymph-flow, and thus decreases cell-output.

These findings are in accord with the theory that makes mechanical factors responsible for rapidly appearing lymphocytosis. They show that there are more such factors than has been supposed. Especially do they indicate that the contribution of cells through the thoracic duct may be important in the production of lymphocytosis, and is not, as is often asserted, subsidiary to direct migration into the blood of cells from spleen, bone-marrow and the lymph-glands.

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THE ENZYMES OF FIBRIN.[1]

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The cells of inflammatory exudates contain two enzymes, one active in an alkaline, the other in an acid medium; the first belonging to the polynuclear leucocytes, and the second to the mononuclear phagocytes (1). The present work is an attempt to determine whether there is a resemblance between the enzymes of fibrin and those of inflammatory exudates in their behavior toward acid and alkali.

Fibrin has been long classed among the changed proteids, one of the chief characteristics of which is insolubility, and yet nearly a century ago it was observed by Arnold and by Berzélius (quoted by Arthus (2) and also by Dastre (3)) that fibrin was soluble in the presence of ammonium chloride. Later experimenters, especially Denis (4) in 1838, and Limbourg (5) in 1889, investigated many salts, including sodium chloride (Denis, Hammarsten (6), Green (7), Limbourg, Rulot (8)), sodium sulphate (Denis, Limbourg), potassium chloride, potassium iodide, sodium iodide, potassium bromide, ammonium nitrate, ammonium sulphate, magnesium sulphate (Limbourg), potassium nitrate (Denis, Zimmermann (9), Limbourg), calcium sulphate (Green), and aqueous solutions of sodium fluoride (Arthus, Dastre, Rulot). Fibrin was found to be soluble in solutions of nearly all these salts, though in some more readily than in others.

The most extended investigation of the action of acids was made by Fermi (10). He tested the effect of hydrochloric, sulphuric and nitric acid in five per cent. solutions, and of lactic, citric, acetic, butyric, oxalic, malic and formic acids in one per cent. solutions. All had a solvent effect, hydrochloric being strongest, and nitric, sulphuric, acetic and butyric weakest. Incidentally he allowed

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fibrin to stand in water, and found it dissolved, but no importance apparently was attached to this fact.

Alkali, in a five per cent. solution of sodium hydroxide, was tested by Deutschmann (11). This dissolved the fibrin of certain small animals, such as guinea-pigs, rats, mice, in less than an hour; of the larger animals, namely, dog, cat, pig, ox, and man, in several hours. In fact, unless the fibrin was previously heated to boiling, all these substances, neutral salts, acids, or alkalis, dissolved it with varying degrees of ease. Warmth was found to favor the action, while a low temperature retarded but did not prevent it. These facts suggest the action of an enzyme, but this conception has been years in developing; the earlier investigators accepted the phenomenon as a simple solution, the effect of a salt on fibrin.

The species of the animal was found to effect the ease of solution of the fibrin. Zimmermann, in 1846, reported that the fibrin of the ox and calf was insoluble, while that of man was soluble with difficulty. Deutschmann found the fibrin of the smaller animals more quickly soluble than that of the larger. Fermi, who experimented with the fibrin of the pig, sheep and horse found that the pig's fibrin dissolved most easily, the calf's with most difficulty, while fibrin of the horse and fibrin of the sheep were intermediate.

The view that a simple solution of fibrin occurs in the presence of various substances has had many supporters, even to within a few years. Evidence which weakened this theory was afforded by analysis of the resulting solution: two coagulable proteids were found by Green, one coagulating at about 56° C. and the second at about 60° C. Other investigators (Hasebroek (12), Herrmann (13), and Limbourg) found the coagulating point of the second product to be nearer 75° C., but agreed with Green upon the constant occurrence of these two products as a result of the solution of fibrin. Limbourg, in 1889, and Fermi, in 1891, found a third substance non-coagulable and giving the biuret reaction. Limbourg proved it to be a peptone. In 1894, Dastre again called attention to the formation of this third product, a soluble proteid, incoagulable by heat, and shown by the biuret test after the removal by heat coagulation of the two coagulable proteids previously mentioned. Dastre found this third product to consist of pro-peptones

and peptones, and its presence caused him to consider the disappearance of the fibrin a chemical alteration, a true digestion, and to suggest once more the possibility of a soluble ferment either derived from the blood and adhering to the fibrin, such as pepsin or trypsin (the two latter being the only two soluble proteolytic ferments generally known at that time) or introduced into it by putrefactive organisms.

Many believed that the decomposition of fibrin was due to the putrefactive organisms, but this possibility was disproved by Green (9), who conducted his experiments at a temperature only a little above freezing, and in a ten per cent. sodium chloride solution, too strong a solution to favor the putrefactive organism; and by Dastre, who took every precaution to exclude microörganisms and used a fifteen per cent. sodium chloride solution and a two per cent. sodium fluoride solution. Other observers have prevented development of microörganisms by a variety of substances, such as chloroform, alcohol, phenol, ether, thymol, hydrocyanic acid and toluol.

Putrefactive organisms being excluded, a possible enzyme was still to be found. Dastre discredits the idea that pepsin is present in amount sufficient to digest fibrin, for otherwise pepsin could always be found in considerable quantities in the blood, and its presence cannot be demonstrated. Moreover, pepsin must act in an acid medium; two per cent. sodium hydroxide destroys pepsin, but dissolves fibrin. Neither, Dastre adds, can it be trypsin, because trypsin breaks down fibrin into true peptones, with formation of tyrosin; but no matter how long fibrin remains in salt solution, no tyrosin is produced.

Though Friedrich Müller (14) had demonstrated in 1888 the presence of a proteolytic enzyme in the leucocytes, its part in the solution of fibrin was not investigated until Rulot proved in 1904 that the digestion of fibrin was caused by leucocytes imprisoned in the meshes of fibrin. Rulot's work is particularly satisfactory because it is so conducted as to control the presence or absence of leucocytes, and to measure the results of digestion, not macroscopically, but more accurately, by the Kjeldahl determination of nitrogen. In order to get pure fibrin, free from leucocytes, he used two methods. In one he prevented the clotting of the blood

by the addition of sufficient concentrated salt solution to make the whole volume of blood a four per cent. solution of sodium chloride. The blood was allowed to settle and the supernatant plasma was pipetted off and filtered. When diluted to about four times its volume with water at about 50° C., fibrin was formed. In the second method of obtaining pure fibrin coagulation was prevented by injecting a solution of pro-peptone into the external jugular vein; from the plasma after centrifugalization and filtration, fibrin was formed by a current of carbon dioxide. This pure fibrin was very nearly insoluble in saline solutions, but the same fibrin taken from unfiltered plasma and having added to it the superficial layer of corpuscles, containing great numbers of leucocytes, digested rapidly, with the formation of peptones and pro-peptones, which were rarely found after the very slight solution taking place with pure fibrin.

Rulot's work has so well established the fact that disappearance of fibrin in salt solutions is due to the action of a proteolytic enzyme present in the leucocytes, that the present work is devoted to a study of the effect of acid and alkali on the digestion of fibrin, with a view to testing the identity of the enzyme or enzymes present with those already demonstrated in the leucocytes of inflammatory exudates.

Methods.—The methods used have been two. First, solution of fibrin, observed by a macroscopic examination of fibrin suspended in acetic acid and in sodium carbonate of concentration from 0.1 per cent. to five per cent., and in neutral solutions. Macroscopic evidences of the progress of solution are the disappearance of the fibrin, the turbidity and final clearing of the solution, though the last two factors are of slight importance. The time required for the solution of fibrin is a rough indication of the activity of self-digestion. The presence of peptones or albumoses in solution was shown by the buiret test, after the removal of the coagulable proteids by precipitation with trichlor-acetic acid and filtration, the alkaline solutions being neutralized before the addition of the acid.

The second method, which is far more reliable, is the determination by the Kjeldahl method of the nitrogen in soluble incoagulable substances formed from fibrin. This method was used first to

measure the degree of autolysis of fibrin alone. The small figures representing nitrogen in incoagulable substances obtained after solution of fibrin were apparently due to the limited amount of proteid (fibrin) upon which the enzyme might act. To gain an idea of what the enzyme was capable, it was thought desirable to furnish additional proteid, such as heated serum, which a proteolytic enzyme could attack. The figures obtained were much greater than with fibrin alone.

Fibrin was prepared in the ordinary way, by whipping freshly drawn blood. The fibrin was then washed with running water for several hours and dried by squeezing in sterile gauze. For the macroscopic study of solution, 0.1 gram of fibrin was added to each test-tube in which the total volume of liquid was five cubic centimeters, and incubated for four days at a temperature of 37° C.

For the experiments in which digestion was measured by the Kjeldahl method, 0.3 gram of fibrin was added to flasks, each of which contained a total volume of twenty-five cubic centimeters, five cubic centimeters of heated serum, acetic acid or sodium carbonate varying from 0.2 to five per cent. and sodium chloride solution (0.85 per cent.) to make up the required volume. These flasks were then incubated for five days at 37° C.; the contents was coagulated by heating in the water bath, after addition of magnesium sulphate and acidifying with a one per cent. acetic acid solution. The contents of the flask were neutralized by a one per cent. sodium hydroxide solution, and brought to the boiling point over the flame. The incoagulable proteid was then filtered directly into Kjeldahl flasks, the coagulum being washed repeatedly, digested with sulphuric acid and distilled. The amount of nitrogen is expressed in cubic centimeters of $\frac{1}{10}$ N sulphuric acid.

Fibrin dissolved to a considerable degree in all strengths of alkali (sodium carbonate) from 0.1 to five per cent., the buiret reaction being more marked after solution in the lower strengths from 0.1 to 0.5 per cent.

In acetic acid, fibrin dissolved somewhat in strengths from 0.1 to four per cent., very little, if at all, in a five per cent. solution. The biuret reaction after coagulation with trichlor-acetic acid, was more marked in the weakest acid, that is, from 0.1 to 0.4 per cent.

The strongest buiret reactions were obtained when fibrin was incubated in neutral media and in 0.1 and 0.2 per cent. solutions of carbonate. The biuret test was not always strongest when the disappearance of fibrin had been greatest, though this relation existed as a rule. Observation of solution, with the biuret test as a measure of incoagulable proteid, was very unsatisfactory. The nitrogen determinations by the Kjeldahl method gave far more decisive results.

In proof of the fact that the enzyme of fibrin acts on foreign proteid as well as on fibrin itself after incubation at 37° C. during five days, the following experiments are given, the amount of nitrogen of the incoagulable proteid being expressed in cubic centimeters of $\frac{1}{10}$ *N* sulphuric acid.

TABLE I.

The action of acid and alkali on fibrin alone, and on fibrin + heated serum.

	Experiment.	Control.	Acetic acid.			Neutral.	Sodium carbonate.		
			5 per cent.	2 per cent	0.2 per cent.		0.2 per cent.	2 per cent.	5 per cent.
Fibrin ...	*a.*	0.35	1.35	1.6	2.55	2.9	—	—	—
	b.	0.9	—	—	—	5.45	2.6	3.5	3.4
Fibrin + serum..	*a.*	1.9	3.45	6.7	3.7	15.7	—	—	—
	b.	2.9	—	—	—	19.6	25.6	9.25	11.1

Figures obtained when fibrin undergoes autolysis are much less than those obtained with fibrin and serum; the excess is referable to decomposition of proteid of the serum.

TABLE II.

Fibrin + heated serum, in presence of sodium carbonate.

Experiment.	Control.	Neutral.	Sodium carbonate.				
			0.2 per cent.	0.5 per cent.	1 per cent.	2 per cent.	5 per cent.
1	2.00	16.35	14.1	2.8	3.25	—	—
2	1.95	15.70	1.95	2.6	3.5	—	—
3	2.90	19.60	25.6	—	—	9.25	11.1
4	2.35	18.65	20.35	4.8	4.2	12.15	7.85
5	2.30	11.3	12.6	4.2	4.25	5.2	8.05
6	1.95	12.7	5.7	5.2	6.3	—	—
7	2.05	9.65	2.55	2.9	3.9	6.25	7.55

Digestion Caused by Fibrin in the Presence of an Alkaline Medium.—The following table shows the action of fibrin on heated serum, in the presence of an alkaline medium.

These nitrogen determinations show that in whatever strength of carbonate digestion proceeds, there is always some production of an incoagulable proteid. Experiments 4 and 5 were performed with the same fibrin and serum; in Experiment 5, digestion was stopped at the end of twenty-four hours, while in Experiment 4 it was allowed to continue for five days, the period allowed for digestion in the other experiments. Judged by the macroscopic disappearance of the fibrin and by the clearness of the solution there was about as much proteolysis at the end of twenty-four hours as at the end of five days, while the Kjeldahl method showed a decided increase after the longer period.

While in the majority of experiments digestion in 0.2 per cent. carbonate has been greater or approximately equal to that in neutral solution, in several experiments no digestion occurs in the 0.2 per cent. carbonate, though digestion in neutral solution has been active. It has not been possible to explain these exceptions. The same divergence is found in the following experiments, in which (save in the last) washed polynuclear leucocytes from sterile pus produced by repeated intrapleural injection of turpentine, have acted upon heated blood serum. In the last experiment leucocytes were obtained from an abscess produced by subcutaneous injection of turpentine.

TABLE III.

Cells from purulent exudates + heated serum.

Control.	Neutral solution.	0.2 per cent. carbonate.
2.6	15.00	7.75
2.2	13.95	4.8
2.65	14.95	13.75
3.2	22.75	27.95
3.55	19.35	21.35
2.9	15.95	20.9
3.45	16.25	22.45

The foregoing experiments with fibrin (Tables I and II) demonstrate the presence of an enzyme which acts either upon fibrin or upon foreign proteids, such as those of heated blood serum, in the

presence of a neutral or very weakly alkaline reaction. A strength of alkali greater than 0.2 per cent. sodium carbonate is unfavorable to the action of this enzyme and inhibits it. By increasing the strength of alkali to from two to three per cent. sodium carbonate there is an increase of incoagulable nitrogen-containing substances which doubtless are not referable to enzyme action, and perhaps are formed by action of the stronger alkali upon proteid. Incomplete coagulation in the presence of a large quantity of sodium carbonate, which is accurately neutralized with difficulty, may explain part of the increase of nitrogen obtained in the presence of the higher percentages of alkali.

Digestion in the Presence of Acid.—The amount of nitrogen produced by autolysis of fibrin in the presence of acetic acid is given in the following table.

TABLE IV.

Autolysis of fibrin, in presence of acetic acid.

Experiment.	Control.	Neutral.	Acetic acid.				
			0.2 per cent.	0.5 per cent.	1 per cent.	2 per cent.	5 per cent.
1	0.4	—	2.05	2.35	2.4	—	—
2	0.75	4.2	2.65	2.05	1.95	—	—
3	0.35	2.9	2.55	—	—	1.6	1.35

The table does not give evidence that a ferment capable of digesting in the presence of weak acid is present in any considerable amount. In two experiments solution with acid was greatest in the presence of 0.2 per cent. acetic acid and may have been referable to enzyme action. The figures obtained are so small that definite conclusions are not possible.

TABLE V.

Fibrin + heated serum, in the presence of acetic acid.

Control.	Neutral.	Acetic acid.					
		0.2 per cent.	0.5 per cent.	1.0 per cent.	2.0 per cent.	3.5 per cent.	5.0 per cent.
1.6	23.00	9.5	—	12.2	13.65	—	8.55
2.35	18.65	5.8	7.75	7.30	8.15	—	4.50
1.95	15.70	3.7	—	—	6.7	—	3.45
1.95	18.8	3.95	—	4.6	5.75	—	—
2.3	11.3	4.0	3.95	3.95	4.05	—	3.55
1.95	12.7	7.7	6.65	5.3	4.9	—	3.5
2.25	21.65	8.2	10.95	—	10.00	8.75	7.15

In the following experiments fibrin was allowed to act upon heated serum in the presence of acid. Since the figures thus obtained are much greater than those with autolysis alone, it must be assumed that the proteid of the serum has been decomposed.

The mononuclear cells of an inflammatory exudate contain an enzyme which digests proteid in the presence of weak acid and fails to digest in the presence of an alkaline reaction. The following experiment, in which fibrin was obtained from the pleural cavity six days after the injection of turpentine, shows the behavior of this enzyme in the presence of various strengths of sodium carbonate and of acetic acid; at this time the enzyme of the polynuclear leucocytes has disappeared from the fibrinous exudate (1).

TABLE VI.

Action of fibrin of an inflammatory exudate on heated blood serum in presence of acid and alkali.

Control.	Acetic acid.					Neutral.	Sodium carbonate.		
	5.0 per cent.	3.5 per cent.	2.0 per cent.	0.5 per cent.	0.2 per cent.		0.2 per cent.	0.5 per cent.	1 per cent.
2.0	5.05	4.85	3.15	8.45	9.05	17.2	4.05	4.5	4.7

Enzyme digesting in alkali (leucoprotease) is present (Table VI), if at all, in very small amount, but the enzyme (lymphoprotease), which digests in acid, is active. The experiment suggests that this enzyme digests well in the presence of a neutral medium. This fact may explain some of the discrepancies previously noted (Table II).

Table VI shows that the enzyme of fibrin of the inflammatory exudate, which is active in 0.2 and 0.5 per cent. of acetic acid, is almost completely inhibited by two per cent. acid. The higher figures obtained with greater strength of acid are probably referable to direct action of the acid upon the proteid used, or to incomplete coagulation as the result of inaccurate neutralization. In all of the experiments with fibrin of the blood figures obtained with 0.2 per cent. of acid are considerably greater than those of the control. That enzyme of fibrin which acts in an alkaline medium is identical, it has been shown, with leucoprotease obtained from

ploynuclear leucocytes of an inflammatory exudate. But since leucoprotease when obtained by treatment of leucocytes with alcohol and ether fails to cause digestion in the presence of acid, that digestion which occurs with fibrin of blood in the presence of acid is explained by assuming the presence of a second enzyme.

When fibrin of blood acts on heated serum in the presence of acetic acid, increasing the strength of the acid produces a corresponding increase in the amount of nitrogen (Table V), the maximum, as a rule, being reached with a two per cent. concentration of acetic acid. Further increase causes an inhibition of formation of incoagulable nitrogen containing substances. This maximum of digestion in two per cent. acid has not been observed with the enzyme of the inflammatory exudate, and is not explained by the available data.

To test further whether the amount of proteid disintegration obtained was due to the enzyme action, or merely to the action of the acid on the proteid, the effect of unheated fibrin and of fibrin heated to 100° C. upon heated serum was tested, and for further comparison the action of acid alone on heated serum.

TABLE VII.

Control experiments.

Experiment.		Acetic acid.					
		0.2 per cent.	0.5 per cent.	1 per cent.	2 per cent.	3.5 per cent.	5 per cent.
a.....	Unheated fibrin + heated serum.	3.95		4.6	5.75		
	Heated " + " "	2.5		3.65	3.45		
	Heated serum.	1.65			1.65		2.4
b.....	Unheated fibrin + heated serum.	8.2	10.95		10.00	8.75	7.15
	Heated " + " "	2.8	2.8		3.7	3.45	4.1
	Heated serum.	2.9	2.95		4.15	4.2	4.3

The experiments with the heated and unheated fibrin show a greater production of incoagulable nitrogen when the enzyme has not been destroyed by heating, while those with heated serum suggest that the acid itself in strength from two to five per cent. causes disintegration of fibrin and of proteid, increasing with the strength of the acid.

Conclusions.—(*a*) There is present in fibrin an enzyme which acts in a neutral and slightly better in an alkaline medium, thus resembling the enzyme present in the polynuclear leucocytes obtained from an inflammatory exudate. This enzyme acts not only on fibrin, causing autolysis, but upon foreign proteid (coagulated blood serum) as well. The action of this enzyme is inhibited by increasing the strength of the alkali above 0.2 per cent. sodium carbonate.

(*b*) Fibrin contains an enzyme which acts in the presence of a weak acid. This enzyme acts upon foreign proteid as well as upon fibrin itself and is probably identical with the similar enzyme which occurs in the large mononuclear cells of an inflammatory exudate.

I wish to acknowledge my indebtedness to Dr. Opie for suggesting this problem to me, and for his assistance and oversight during the course of the work.

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BONE FORMATION IN SCLEROTIC ARTERIES.*

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Plates XXIV and XXV.

In recent years a number of papers have appeared tending to show that the formation of bone in arteries is not as rare as has been generally supposed. In the large vessels in six cases of arteriosclerosis, Mönckeberg was able to find sixteen osseous foci. The older literature seems to indicate that the aorta and heart valves are the favorite sites for ossifying processes in the cardio-vascular system. Mönckeberg's contribution, however, has clearly demonstrated that not only the aorta, but also vessels of the extremities, such as the femoral, the anterior and posterior tibial arteries may not infrequently be involved.

While engaged in a study of the pathology of vessels in gangrene of the lower extremities, we encountered rather extensive areas of ossification in the arteries of two limbs, both obtained from patients afflicted with advanced arteriosclerosis. We wish to record the histological features presented by one of the cases, to point out how these differ from what has been observed by other authors and to discuss in brief the theories that have been advanced in explanation of heteroplastic bone formation.

Our case was particularly interesting because of the large number of bony plaques and because so many of the larger arteries of the extremity were affected. Either true bone or osteoid tissue was found distributed throughout their length in the following arteries: the anterior and posterior tibial, the peroneal, and the plantar; in short, in all of the five vessels examined except the dorsalis pedis.[1] A perusal of the literature failed to reveal any record of bone for-

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[1] We are unable to state the extent of the areas of ossification because we made no serial sections; but in one instance, as was seen in longitudinal section, the osteoid patch extended the entire length of the piece, namely, 3 mm.

mation in either the plantar or peroneal vessels and our case seems therefore to be unique in the distribution of the lesions.

The arteries removed for examination were dissected out in continuity from the limb. The macroscopic lesions of interest may be embodied in a brief description of the extremity, as follows: The left leg has been amputated 15 cm. above the knee joint. The second toe is greenish black, dry and mummified. On the inner side of the big toe is a large bulla filled with serum and blood. The anterior tibial artery shows typical changes of arteriosclerosis in various stages; numerous patches of atheroma, white areas of thickened intima and calcification. In places there is a large accumulation of atheromatous tissue which presents itself on section as a crescentic obturating mass and leaves but a small portion of the lumen of the vessels patent. The dorsalis pedis artery is similarly affected. The posterior tibial and peroneal arteries are also sclerotic; in some parts they are patent, in others filled by recent thrombi. The plantar arteries are of the pipe-stem variety. The lesions, in short, are those of advanced arteriosclerosis with calcification.

A number of pieces were removed for histological study from each of the following arteries: anterior and posterior tibial, dorsalis pedis, peroneal and plantar. The tissues were decalcified with 5 per cent. nitric acid in the usual way, imbedded in celloidin and stained with hematoxylin, with hematoxylin and eosin, by Van Giesen's method, with Unna's polychrome methylene blue, with orcein, and with Weigert's elastic tissue stain. Special methods were employed in the search for hemosiderin and amyloid changes.[2]

Before describing the histologic picture we may call particular attention not only to the identity of location of the ossifying process in the various arteries, but also to the uniformity in the structure in different situations. Osseous and osteoid tissue were only discovered in the middle coat; and the process of bone formation showed but slight variations from a fixed type. We will therefore discuss in detail the most common appearances, namely those exemplified in many of the sections of the posterior tibial artery, and

[2] The test for amyloid proved negative; so that the presence of amyloid, which has been pointed out by Poscharissky as important, does not seem in our case to be essential to ossification.

only mention in brief any deviations or peculiarities encountered elsewhere.

Histology.—The most striking pathological changes may be classified under the following groups: (1) the lesions of arteriosclerosis; (2) the lesions dependent upon thrombosis; (3) the lesions belonging to the ossifying process.

1. *The Lesions of Arteriosclerosis.*—The arteriosclerotic changes involve both the intima and media. In the intima we find the usual proliferative process, varying considerably in degree in the same and in different vessels. Wherever the lesions are advanced, degeneration and atheroma are also to be seen. The media of almost all the vessels is the site of calcific deposits, either in the shape of lime plates which occupy a large part of the circumference of the artery, in the form of irregular nodular plaques representing calcified atheromatous areas, or as a diffuse infiltration of the media with lime salts (see Fig. 1).

2. *Thrombosis.*—Thrombosis with vascularization and connective tissue proliferation in the clot is seen in the posterior tibial (see Fig. 1). The various stages of organization are well represented in different situations along the course of the artery; in some places there is very recent thrombosis, in others the lumen of the vessel is obliterated by well-organized, canalized tissue.

3. *The Ossifying Process.*—This occurs only in the media, and for the most part in the presence of lime (see Fig. 1). Calcific deposits and young connective tissue appear to be the *sine qua non* for the genesis of bony proliferation; we see the lime and connective tissue intimately associated with the process of bone formation in all the specimens studied (see Fig. 2).

Occupying a large portion of the media, in the form of an arc-like band, varying considerably in thickness and extent, we find a zone of young connective tissue. This is composed of numerous capillaries, spindle-shaped cells, lymphoid cells and a small amount of fibrillar substance. It apparently invades, destroys and replaces the muscle fibers. Evidence of the disintegration and substitution of the musculature is furnished by degenerate muscle cells without nuclei, by nuclei which fail to stain, and by reactive changes on the part of the muscle. These last are multiplication of the nuclei,

the formation of muscle giant cells and the conversion of many muscular elements into strands of connective tissue.

The young vascular fibrous tissue communicates at frequent intervals with the adventitia by means of well developed smaller or larger capillaries. These penetrate the media obliquely in cross-sections of the arteries and are often seen surrounded by a few round and spindle-shaped cells. As regards the constitution of the young connective tissue certain variations may be observed; the differences are mainly in the vascularity, density or fibrous nature, and in the presence of lymphoid or round cells. Wherever the process is an old one we are apt to encounter tissue rich in fibrous elements, and this sometimes in the vicinity of well developed and maturing bony tissue; on the other hand, there are many areas made up of round cells infiltrating the media in streaks, or larger, broader conglomerations which probably represent very early stages in the origin of the connective tissue above described.

The Osseous and Osteoid Tissue.—Where the young connective tissue comes into contact with a lime plaque a conversion of a portion of the latter into osteoid tissue sometimes occurs. Thus we find large portions of some of the lime plaques altered into a homogeneous substance which stains well with eosin and in which a few or a moderate number of nucleated elements are included. It is most frequently seen along the outer margins and at extremities of the calcareous plaques as a narrow strip bordering a plate of lime or as a peninsular projection capping a calcareous plate (see Fig. 3). Close scrutiny makes it evident that this new tissue always substitutes an area previously occupied by lime, in other words, represents a transformation of part of a lime plaque into a peculiar new substance—the ground substance of bone. A very sharp, notched line with the concavities toward the ground substance of the bone separates this from the lime. On the other side there is connective tissue varying somewhat in its architecture. It is in such places, then, that a deposition of osteoid tissue occurs *pari passu* with an absorption or erosion of the calcified media.

All the stages from the youngest variety of osteoid tissue up to true bone can be traced in the sections. Where the process is recent, the number of included cells is small; the nuclei of the cells

are large, and signs of compression, shrinkage and alteration in cellular outline are not manifest. In the older types we see a gradual change into true bone corpuscles. Between the young and the old variety an interesting series of cytomorphic changes may be seen. The ground substance, too, although for the most part of the "osteoid" type, shows varying degrees of calcification, and is in places practically indistinguishable from true bone matrix.

That side of the osteoid or osseous patch which is opposed to the lime plate is applied directly to the osteogenetic connective tissue. Nowhere, however, are typical osteoblasts to be found; in place of these there are flattened endothelial cells, or larger elements of the fibroblast type. Such cells are actively engaged in a double process, one, bone resorption, another, the deposition of either bone matrix or osteoid substance.

The Process of Resorption of Calcific Material.—Resorption of calcific material is evidently an essential stage in the production of bony tissue and is effected in main by the action of giant cells and by the young connective tissue cells themselves (see Figs. 4 and 5). That still another modus can play a rôle in this process is seen in a number of places where capillaries appear to empty their contents along one margin of a lime plate. In such places, a notched border bears testimony to the fact that resorption is taking place. Sometimes large blood sinuses adjoin the lime plates, particularly where marrow spaces are being formed (see Figs. 2 and 4).

This resorptive metamorphosis is usually accompanied by a deposition of osteoid tissue, corresponding in extent and conformation to the areas previously occupied by lime (see Fig. 3). Here and there, however, large bays have been produced in the ends of lime plates by the invasion of young connective tissue without any vestige of osteoid or osseous tissue (see Fig. 5), and the old site of the lime plates may be occupied by the persisting young connective tissue or by the marrow cavities which are being formed.

The most advanced resorptive changes—and with these the most marked osteoplasia—occur at the extremities of the lime plates. Here the connective tissue cells lying in concavities or notches of the lime patches seem to invade and cause the disappearance of the calcific material. When a band of osteoid tissue intervenes between

osteogenetic connective tissue and the lime plate, the same resorption takes place except that it goes hand in hand with a deposition of osteoid substance. It is in such areas that the wide sphere of influence exerted by the osteogenetic tissue becomes notable. Separated from the lime by newly deposited ground substance, the cellular elements still manifest their power of inducing active resorption.

The Formation of Marrow Spaces.—Where the transformation of the lime plates into osteoid or osseous tissue has evidently been of long duration, and where the trabeculæ of bone matrix include larger or smaller areas of young connective tissue, there we may speak of the production of marrow spaces. In such places the connective tissue usually shows large dilated capillaries and blood sinuses. The former are filled with blood, but show no bone-marrow cells. The latter adjoin eroded lime plates or lie between such plates and bands of osteoid tissue. They contain red and white blood cells and a few giant cells resembling osteoclasts. The picture presented gives the impression of an unfinished stage in production of bone marrow. However the elements of true bone marrow are absent; and we have here rather the result of the inclusion of osteogenetic connective tissue between bony trabeculæ than the primary production of true marrow.

Giant Cells.—Giant cells of various types are found scattered throughout the media where the latter shows invasion by young connective tissue. Usually they lie in concavities of the lime plaques which they are apparently engaged in resorbing. Others are seen where there is no lime and still others where there is a very faint pulverous infiltration with calcium salts. As a rule they have rather large irregularly shaped bodies which stain well with eosin, and show small ovoid nuclei situated either centrally or scattered without definite arrangement throughout the cell body. Here and there a giant cell of bizarre form appears to be almost a continuation of a lime spicule; for the line of demarcation between calcium and body of the cell is incomplete (see Fig. 5). Such cells remind one of osteoid tissue. Some of the giant cells have very long bodies that seem to be adapted to the concavities of the lime plates in which they lie.

Variations in Histological Picture.—A very few sections showed the following deviations from the usual type above described: the construction of Haversian canals, the formation of osteoid tissue by a modification of the collagenous fibrils, and finally small areas resembling embryonic cartilage.

Fig. 6 shows a longitudinal section of the posterior tibial artery with one complete Haversian canal, another in the making. Here, too, there are indications of concentric disposition of the bone cells, and the apposition of new bone in the normal fashion.

A number of specimens exemplified a more unusual type of osteogenesis by transformation of connective or possible degenerate muscle fibers adjoining a lime plaque into strands of bone matrix. This may be seen in Fig. 7, where a tongue-like process of osteoid tissue has its origin in fibrous tissue quite independent of the neighboring calcific patch. Here, too, the young ossifying connective tissue plays a rôle, for it can be traced directly into the newly formed matrix, in whose production it is evidently engaged.

Finally, interesting foci of cartilage similar to the embryonal type were found in the plantar arteries. Such areas replace calcified atheromatous nodules and are made up of large polygonal or spheroidal cells, at times grouped in twos or fours, at times in the shape of larger conglomerations of cells with varying amount of intercellular substances. Cells not unlike these were seen in calcified atheromatous patches and at the extremities of calcified plaques in other arteries. Such cells are usually surrounded by a well-defined, somewhat refractile layer; and now and again appear to take part in laying down a homogeneous substance similar to the usual osteoid matrix.

Summarizing our anatomical findings we may say that we are dealing with an exquisite example of extensive bone formation in practically all of the larger arteries of the lower extremity; that the process is evoked in the media by the activity of a young connective tissue, the original elements of which apparently have migrated from the adventitia in the shape of vascular sprouts; and that where the proliferating connective tissue comes into contact with calcium salts, either true osteoid tissue or true bone is elaborated. The osteoid tissue arises in a manner analogous to the

origin of true bone; sometimes strands of the ground substance of bone appear in the connective tissue and merge into the collagenous fibrils as if they were a modification of these; and more frequently osteoid material is formed in lime plaques. The process is therefore for the most part not unlike that of endochondral bone formation. The lime plates of the media may be said to correspond to the calcified cartilaginous matrix of the long bone; the penetration of a blood vessel or bud from the periosteum into the calcified cartilage finds its parallel in the invasion of the media by the young vascular tissue; the disintegration of the calcified cartilaginous substance, the lodging of an osteogenetic layer of so-called osteoblasts, the appearance of osteoclasts are simulated. The progressive differentiation of the connective tissue cell into a true bone cell with canaliculi is also imitated. It is only in the absence of true primary marrow spaces which develop in the embryonal bone that we find a marked deviation from the normal process.

The occurrence of newly formed bone outside of the cardiovascular system is not at all infrequent. The following sites have been recorded in the literature: pia and choroid (Virchow), adductor muscles (Ponfick), bladder mucosa (Morpurgo), laparotomy scar (Askanazy), lung (Pollack, Poscharissky), pleura (Laboulin, Hurtado and Pollack), dura (Cruveillhier), eye (Pagenstecher, Klebs, Knapp), stomach (Minkiewicz), liver (Cornil, Ranvier), and lymph nodes (Pollack and Poscharissky). In an examination of the lungs from one hundred autopsies, Pollack was able to demonstrate the presence of osseous nodules in sixteen cases; and Poscharissky, limiting his search to calcareous foci, succeeded in demonstrating bone in fully 60.7 per cent. of the foci examined.

When we compare the frequency of these findings with the relatively small number of reported instances of bone production in arteries, we can not but believe, with Mönckeberg and Bunting, that a more thorough investigation of sclerotic arteries of the lower extremity would substantiate the view that the process is not at all uncommon. Whereas, before the year 1901, we had only the few isolated examples recorded by Howse, Marchand, Paul, Cohn, Kryloff, v. Schrötter and Röhmer, since then the publication of Mönckeberg's paper, confirmed by our own observations, has en-

larged the number of cases and has changed our notion as to their rarity.

If we now direct our attention to the histological features of the process, we find that, although certain essentials in the production of osseous tissue are regularly seen in all the cases, quite a variety of deviations from normal physiological osteoplastic changes have been described by different authors. Most observers are agreed in the opinion that lime and a vascular young connective tissue are most frequently engaged in, and apparently directly responsible for, the changes leading to heteroplastic bone formation. Bone without marrow and bone with true fatty, or even with red, cellular marrow, have been encountered. Sometimes the so-called osteoid tissue preponderates, at other times progressive metamorphosis into mature bone is evident. Thus Bunting found bone enclosing spaces in which a delicate reticulum, fat cells and cells of bone-marrow type gave the picture of true red marrow. In fact practically all the elements of red marrow were represented. In his case the site of the process was a sclerotic aorta; the intima was penetrated by vessels surrounded by young connective tissue and showed production of cancellous bone at points where the connective tissue found its way into lime plates.

The minute and carefully detailed description given by Mönckeberg in his paper is particularly illuminating and interesting from our standpoint because our findings so nearly correspond with his. In his arteries, too, there was an invasion of the media with vessels which gave rise to the appearance of young connective tissue, due, in his opinion, not only to a proliferation of the newly immigrated elements, but also to a hyperplasia of the preëxisting connective tissue cells of the media.[8] He considers the presence of lime and of young connective tissue and the process of resorption, the essential factors underlying the genesis of the bone. His view of the sequence of changes that occur may be thus summarized *seriatim:* (1) The immigration of vessels into the diseased vessel wall. (2) The production of a loose tissue, rich in cells, around the vessels and in the vicinity of lime plates. (3) Lacunar resorption of lime

[8] Pollack, too, believes that the old scar tissue rather than new granulation tissue forms osteoid tissue and bone by metaplasia.

and hyaline tissue by virtue of the activity of the newly formed tissue especially of certain pigment cells. (4) Lodging of connective tissue cells along the borders of lime areas with deposition of bone ground substance, inclusion of the cells and their transformation into true bone corpuscles. (5) Conversion of the osteogenetic tissue secondarily into marrow. Inasmuch as the initial stages of the process show no primary marrow formation, Mönckeberg holds that we are not justified in speaking of the presence of true osteoblasts; indeed we should regard the ossific changes as being produced by a metaplasia of connective tissue.

Quite a different view has been propounded by Röhmer who declares that the formation of primary marrow spaces marks the inception of the ossification. There is first a lymphoid tissue, then the building up of fatty marrow, the peripheral cells of which become osteoblasts and form bone out of lime. Cohn, from a study of calcareous plates in the aorta and in the heart valves, concurs in this conception.

In a scholarly paper on bone formation Bunting has recently discussed at length the various theories that have been advanced in explanation of the very interesting phenomena of heteroplastic bone formation in arteries. He showed that Cohnheim's theory of embryonal displacement was not applicable, and suggests two views, either of which may obtain: first, that there is a direct metaplasia of connective tissue into bone after the manner of callus formation, and second, that the calcific material is eroded with the formation of vascularized spaces containing young connective tissue cells, some of which take on the function of osteoblasts and lay down bone, some of the osteoblasts becoming included and forming the bone corpuscles.

Whereas the advocates of the "metaplasia theory" argue that a change of connective tissue into osseous tissue may occur, their opponents wish to show that bone can only be formed in connection with cartilaginous or osteogenetic layers, or from some displaced embryonal bone or cartilage matrix. Von Hansemann and Ribbert are of the opinion that metaplasia rarely comes into play. Whereas Lubarsch and Pollack, finding bone in almost every calcareous nodule of the lung examined by them, think that it arises by meta-

plasia from connective tissue, von Hansemann believes that it has its origin in islands of perichondrium or cartilage. Whatever the conditions may be in other organs, the bone formation in arteries must certainly be explained on the basis of a theory of metaplasia. It would be a work of supererogation to dilate at length upon this phase of the subject; and we will therefore confine ourselves to the conclusions which we have been able to draw from a study of our own cases and from the consideration of the findings of other authors.

It is generally conceded that the presence of lime and young connective tissue is essential to heteroplastic bone formation.[4] The young mesoblastic elements evidently are engaged in the deposition of osteoid substance. The following questions then arise: What influences are exerted by the lime? What causes the appearance of the connective tissue? The intimate association of the lime with the osteogenetic tissue makes it more than likely that the calcium exerts some stimulus upon the young connective tissue which is possibly effective in producing a transformation from the undifferentiated mesoblastic cell into an element comparable in its function to the so-called osteoblast. Although Mönckeberg refuses to call the cells osteoblasts, because they are not developed in primary marrow, we cannot but attribute to them the same rôle that belongs to normal bone-producing elements. Perhaps the lime has not such an important rôle, but the metaplasia into osteogenetic tissue has already occurred before the young connective tissue comes into contact with it. At all events the presence of the lime is essential inasmuch as its erosion or resorption furnishes the proper condition for the elaboration by the connective tissue cells of a homogeneous ground substance, the matrix of bone.

As regards the invasion of the media by vascular connective tissue, this phenomenon may probably be evoked by a variety of causes. Bunting suggests with Paul, that the fracture of lime plates calls forth this immigration of vessels and proliferation of tissue. In our specimens, however, this was not the case; for although old fractures of lime plates undergoing consolidation could

[4] The fact that most of the osteoid tissue lies along the outer margins of the calcified plaques, namely, where the incoming vascular young connective tissue must first meet the lime, is significant.

be observed, such places were neither the seat of young connective tissue infiltrations nor could any bone formation be discovered in their vicinity. In our studies on diseased vessels we have encountered vascular and connective tissue proliferation in the media in the following conditions: as a reparative process, as a compensatory process and as a manifestation of inflammatory change. Thus we frequently find invasion of the media in attempts at organization of thrombi, in the production of collateral circulation by canalization and vascularization of obliterating intima and in disease of the middle coat itself. The most extensive proliferative changes and the most marked instances of ossification seem in our cases to occur in the vessels which are the site of either organizing thrombi or vascularized obliterating thickenings of the intima. These circumstances would seem to point to the view that perhaps the primary entrance of vessels in the media is incited by the changes incident to thrombosis and vascularization of the intima. And further that when the new tissue comes into contact with the lime, certain new influences come into play which cause a renewed activity, a transformation into osteogenetic tissue with the consequent production of true bone. Be this as it may, we cannot ignore the presence of the lime plates themselves as factors in the connective tissue invasion as such, all the more so as now and then connective tissue may be present without vascularized obliterating intima or without canalized thrombi. Doubtless all of the causative factors mentioned may exert their part in the process.

It is of interest in this connection to call attention to the recent work of Harvey who was able to induce bone production in the aorta of rabbits by the application of irritants to the vessel wall. After painting the outer surface of the aorta in some cases with a three per cent. solution of silver nitrate and in others with a two per cent. solution of cupric sulphate, bone with Haversian canals, with bone marrow or bone alone, or osteoid tissue developed, in areas of calcareous degeneration, in a number of rabbits after about two to six months had elapsed. Harvey accepts the hypothesis that there is a metaplasia of connective tissue, that is, a direct conversion of young fibrotic tissue into bone with possibly an intermediate step of cartilage-like tissue formation.

In our own cases we meet with two types of metaplasia: one is evidenced in the young tissue which lays down the ground substance of bone in eroded or absorbed lime areas; the other is shown in those elements that cause the deposition of ground substance in preëxisting connective tissue. In a very few places there are suggestions of cartilage production; but this cartilage is never transformed into bone.

Summing up our own view of the process we may say that by virtue of some stimulus, be it an organizing thrombus, an attempt at vascularization of obliterating pathological intima, or possibly the presence in the diseased mesial coat of lime alone, a penetration of the media with vessels takes place. This is followed by the proliferation of young connective tissue in the media which comes into contact with the lime; at such points of meeting, the young connective tissue cells manifest a new function by producing the ground substance of true bone.

We wish to express our indebtedness to Dr. F. S. Mandlebaum, director of the laboratory of Mt. Sinai Hospital, for the preparation of the photo-micrographs.

EXPLANATION OF PLATES XXIV AND XXV.

FIG. 1. Transverse section of posterior tibial artery. Lower left hand quadrant shows a lime plaque with bone formation in its right extremity. On the right the media shows a calcified atheromatous nodule. In the upper half there is considerable infiltration of the media with vascular young connective tissue. The lumen of the vessel is obliterated by an organizing thrombus.

FIG. 2. Bone and marrow formation in a lime plaque.

FIG. 3. A portion of the wall of the peroneal artery. The extremities of the dark calcified plaques on the right and left are tipped by osteoid tissue, which takes the place of areas previously occupied by lime.

FIG. 4. Posterior tibial artery (high power). The upper left hand quadrant shows pale osteoid and darker osseous tissue. The greater portion of the photograph is occupied by young vascular connective tissue. Below and to the right there is erosion of a lime plaque by blood and by an osteoclast in a free space.

FIG. 5. A portion of the media of the posterior tibial artery (high power). On the right is the eroded extremity of a lime plaque penetrated by young connective tissue and bordered by osteoclasts.

FIG. 6. Longitudinal section of posterior tibial artery. The dark area on the right is lime. In the concavity of the lime plaque is an annular area of osteoid tissue containing an Haversian canal.

FIG. 7. Media of posterior tibial artery. The dark area on the extreme

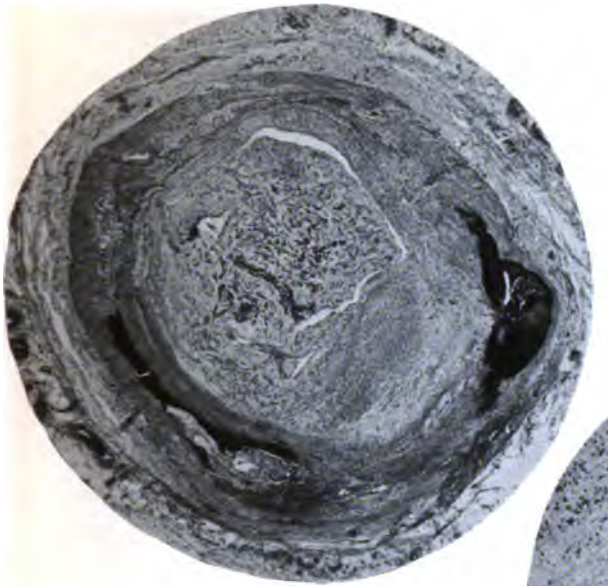

FIG. 1. 3

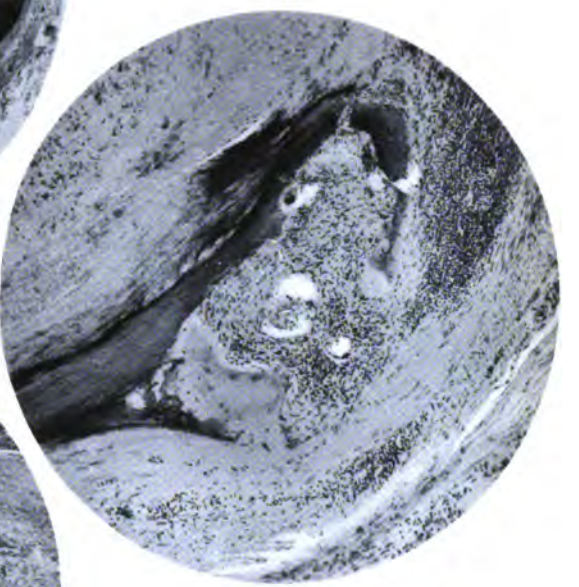

FIG. 2

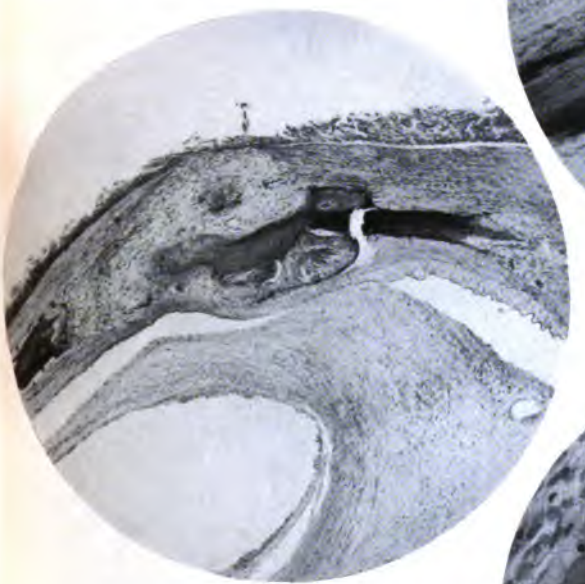

FIG. 3.

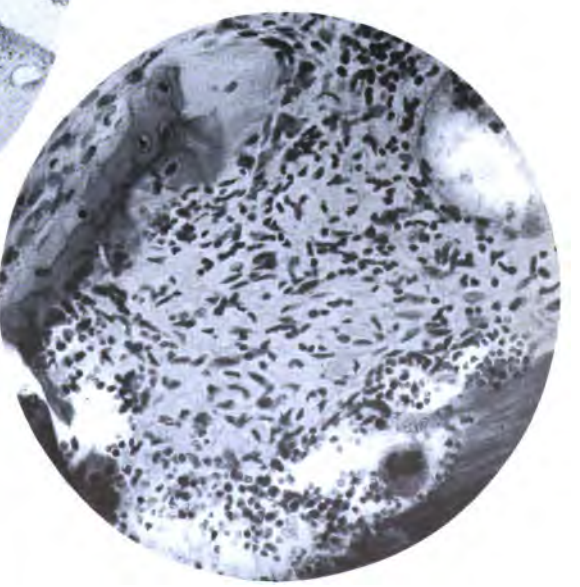

FIG. 4.

FIG. 5.

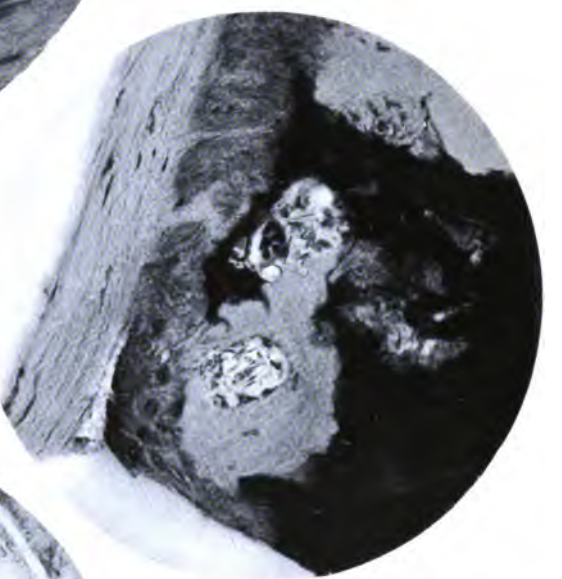

FIG. 6.

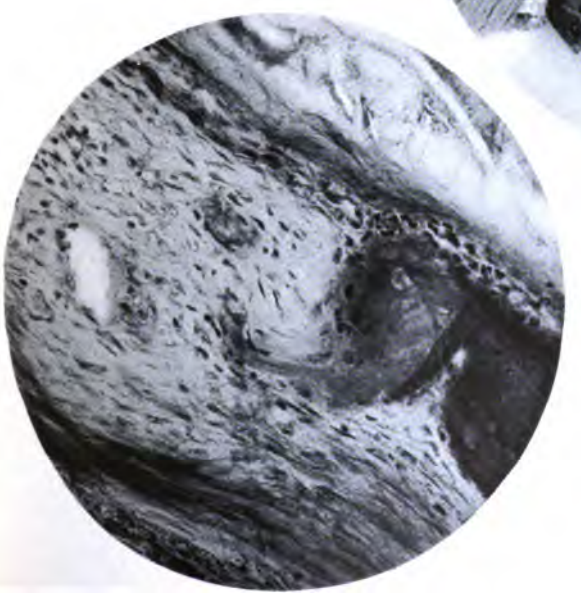

FIG. 7.

right is osteoid tissue which is drawn out into a tongue-like process. This represents connective tissue which has been directly changed into osteoid tissue by metaplasia.

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ANGEIOMATA IN VALVES OF HEART OF A NEWLY BORN CHILD.*

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PLATE XXVI.

The following pathological condition was found in the heart and bladder of a male child eighteen days old, who came to autopsy at the Johns Hopkins Hospital in August, 1898, and is, on account of its rarity, worthy of a brief description.

The mother of the child gave a normal history, and the present pregnancy, her first, was brought to a successful issue in the obstetrical ward of this hospital, without interference or complications, except a few mild epileptiform convulsions before and after delivery.

The child, however, never did well, grew progressively thinner and weaker, and succumbed at the end of eighteen days to constant vomiting and diarrhœa. The weight at birth was 2,800 gm., at death 2,095 gm. Nothing abnormal was noted about the heart sounds in the obstetrical ward.

At autopsy, the body was that of an undersized, newly born, male child, showing much emaciation and considerable livor mortis. There were no special marks upon the external surface. The intestines showed a considerable degree of follicular enteritis. With the exception of the heart and bladder, the other organs presented no apparent pathological alterations.

In the heart the aortic and pulmonic valves were normal; the foramen ovale was still patent by a small opening a few millimeters in diameter. On the tricuspid and mitral valves, however, strung along their upper or auricular surfaces, were from eight to ten minute, dark purplish red globules, varying in size from a pin's point to one, on the tricuspid valve, as large as a pin's head. All of these protruded more or less from the valvular surface, while the larger ones were distinctly pedunculated. They were quite hard

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and tense to the touch, and very firmly attached. On removal with the forceps, these globules burst, and seemed to contain fluid blood. As their situation along the valves was above the insertion of the free edge, they apparently interfered little, if any, with perfect closure.

On microscopic examination the protruding globules are seen to be spaces lined by a definite, single layer of endothelium, and filled with blood (Plate XXVI, *a, a, a*). They are not mere extravasations of blood into the surrounding tissues, but greatly enlarged capillaries or vascular spaces. Extravasations have occurred, however, in a few places as at *b, b, b,* in the figure, but form small interstitial hæmorrhages hardly worth the name hæmatomata. Normal capillaries may be seen, in the section reproduced here, at the points *c, c, c.* The connective tissue of the valve is characteristic of young connective tissue and probably not increased. The apparent thickenings in the figure are produced by the shrinkage and curling of the valve. The delicate free edge is seen folded under the remaining portion, and is unaffected by these growths.

In the bladder, under the epithelium about the trigonum, were three similar but larger growths, nearly the size of a small pea. To the touch these were not tense, but rather flabby. On section they are apparently composed, like the foregoing, of vascular spaces. These are, however, more numerous, and, together with tortuous, enlarged capillaries and considerable ecchymosis, form the small projections over which the epithelium of the bladder is continued.

The chief interest in this article centers in the occurrence of these angeiomata in the heart, which must be very rare or else hitherto overlooked. They probably differ only in their unusual situation from the familiar birth marks, such as angeiomata of the lip, etc., which occur so frequently on the external surface of the body, and also in some of the internal organs. I can find, in the literature at my command, only one instance described of the occurrence of growths of an angeiomatous nature in the heart, whether of infant or adult. This is a case in an adult, recently described by Rau,[1] of a small angeioma under the endocardium of the right

[1] Rau, *Arch. f. path. anat.*, 1898, cliii, 22.

auricle, and which he cites as the first instance of the kind described. Henoch,[2] however, mentions some rare cases of valvular "hæmatomata" described by Luschka[3] and Parrot[4] in very young infants who had died without valvular symptoms. These authors describe similar gross appearances and doubtless refer to the same pathological alterations as those mentioned in the present article, though they speak of them as hæmatomata and not true angeiomata. Henoch considers that they usually become absorbed within a short time, leaving no scar, or one so small as to be of no pathological importance; though, occasionally, even so slight an alteration may become the starting point of an obscure insufficiency in later life, possibly due to interference in the proper nutrition or development of the valve.

EXPLANATION OF PLATE XXVI.

Tricuspid valve, hardened in Zenker's fluid and stained in hæmatoxylin and eosin. The section passes from base to free edge.

a, a, a. Protruding globular spaces filled with blood and lined by flat endothelial cells.

b, b, b. Extravasations of blood into tissue.

c, c. Ordinary capillaries.

d. Free edge of valve curled under main portion.

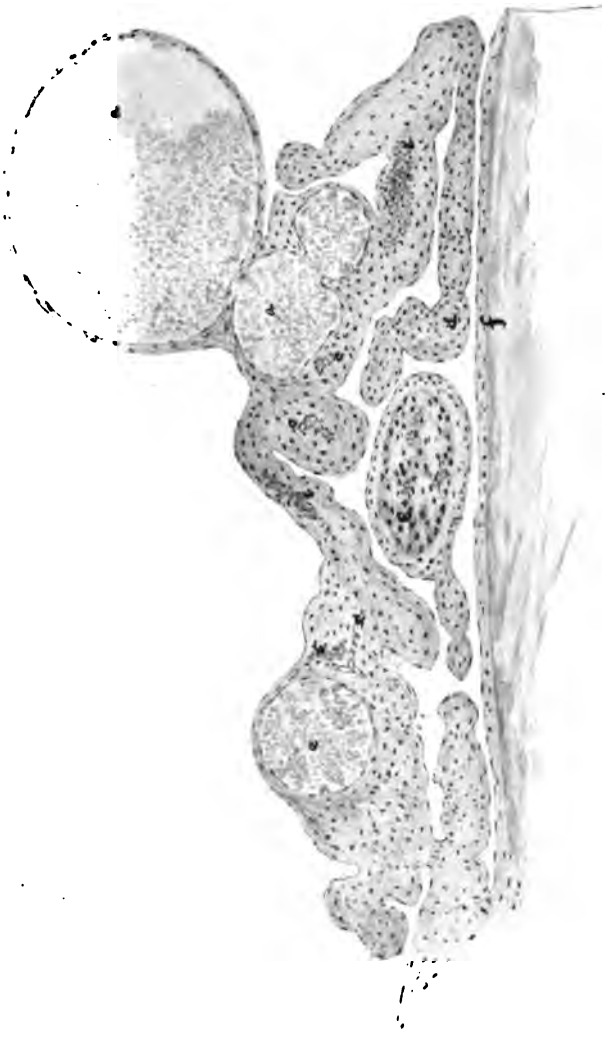
e. Papillary muscles.

f. Wall of ventricle.

[2] Henoch, Vorlesungen über Kinderkrank, 8th ed., Berlin, 1895.

[3] Luschka, *Virchow's Archiv.*, 1857, xi, 144.

[4] Parrot, *Arch. de Physiologie,* 1874, vi, 538.



STUDIES IN RESUSCITATION: I. THE GENERAL CONDITIONS AFFECTING RESUSCITATION, AND THE RESUSCITATION OF THE BLOOD AND OF THE HEART.*

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CONTENTS.

INTRODUCTION.

The work on resuscitation was begun by two of us (S. and G.) more than five years ago, intermitted for a time through external circumstances, and then resumed in conjunction with the third author (P.), who is largely responsible for the preparation of the paper for publication. The original incitement to the work was in part the remarkable results obtained by Kuliabko[1] in the resuscitation of the excised mammalian heart. It was clear at the outset that, if the resuscitation of the heart *in situ* could be accomplished after equally long intervals, the limit up to which the resuscitation of an entire animal could be hoped for must be determined by the power of resistance of organs less tenacious of life and less susceptible of resuscitation than the heart. Our first task, then, was to study the conditions of resuscitation of the heart *in situ,* and the next to fix again, in the light of the results of this study, the limits of possible resuscitation of the least resistant of the systems essential

* Received for publication, January 27, 1908.

[1] Kuliabko, *Arch. f. d. gesam. Physiol.,* 1902, xc, 461.

to life—the central nervous system. Several papers[2] have been published on special portions of our investigations. Our present aim is to give a somewhat general view of the subject, covering all the systems so far studied and embodying new results in the case of those systems already treated of. The results on the central nervous system and on the glands and muscles will be embodied in future papers. Besides the practical purpose of the observations—to contribute something, if possible, to the technique of total resuscitation, which might be useful in the treatment of such accidents as chloroform or coal gas poisoning, drowning and other forms of asphyxia,—we constantly kept in view, particularly in our studies on the resuscitation of individual organs or systems, the possibility that by so treating an organ that its activities are suspended, either wholly or in part, and then watching the gradual or sudden restoration of these activities under the various methods of resuscitation, continued for longer or shorter periods of time, some insight might be gained into the life processes of the organ, and the necessary conditions of its activity. If we knew precisely what had happened to an organ or tissue at the moment when, in consequence of interference with the circulation in it, it had ceased to function, and what happened when, under the influence of the restored blood stream, its function again returned, we should certainly possess a very exact knowledge of the nutritive conditions on which its activity depends. If we knew the difference between an organ whose power of normal function is in abeyance but which is capable of resuscitation, and the same organ, absolutely alike, as it seems to all our ordinary tests, when it has just passed beyond the limit of possible resuscitation, we should not be far from an exact knowledge of the meaning of life.

The experiments of Pictet,[3] to cite only one of the many similar results in the field of general physiology, lend particular emphasis to this point. He found that fish might be frozen solid and afterward recover when thawed out, provided the temperature had not

[2] Guthrie and Stewart, *Science,* 1905, xxi, 887. Stewart, Guthrie, Burns and Pike, *Jour. of Exper. Med.,* 1906, viii, 289. Guthrie, Pike and Stewart, *American Jour. of Physiol.,* 1906, xvii, 344. Guthrie and Pike, *ibid.,* 1907, xviii, 14. Stewart and Pike, *ibid.,* 1907, xix, 328; xx, 61. Stewart, *ibid.,* 1907, xx, 407.

[3] *Arch. des Sciences physiques et naturelles,* 1893, xxx, 293–314.

been reduced lower than —15° C. If, however, the temperature had been reduced to —20° C., the fish would not recover when the temperature was raised again.

A formal definition of resuscitation is by no means easy. The circulation in an animal has been stopped for a time. According to the length of the stoppage, changes more or less serious have occurred in the organs. According to the sensitiveness of the various organs to deprivation of blood, the damage to each has been greater or less. If at any moment the question be asked, whether the body as a whole, or any particular organ is capable of resuscitation, the answer must be that much depends upon the method by which resuscitation is attempted. For example, we not infrequently see that, at a certain stage of anæmia of the head end of an animal, the reëstablishment of the circulation and its long continuance may fail to restore the respiration and the other functions associated with the bulb and the higher parts of the brain, Minute after minute, sometimes even hour after hour, goes by, the heart beating fairly well, the blood circulating steadily, but to all our tests, no ground is being gained. The arterial pressure is too low. If now by artificial means the arterial pressure be raised sufficiently, speedy resuscitation may ensue. In such cases, the animal with the means ordinarily employed would be deemed incapable of resuscitation. In ordinary language, it would be said that its head was dead before the circulation was reëstablished. It is only when, under the influence of the higher pressure, the signs of returning function appear that this verdict is reversed. The resuscitability of the animal in such an instance clearly depends on the perfection of the technique employed to resuscitate it. Ought we to say, in another case where resuscitation had not been accomplished even when, in addition to restoring the circulation the pressure was raised, that the stage had been passed at which restoration was possible and that definitive death had occurred before the renewal of the circulation? Plainly we could not say this, since an improved technique might have led to a successful result.

Another question which may be asked is what constitutes successful resuscitation of a tissue element, an organ or an entire animal? It is easy to reply, a restoration of all the normal condi-

tions, structural and functional. But it is difficult to separate the more from the less important conditions and to say of any given tissue or organ or of the whole animal, at a given period in the resuscitation process, such and so many functions have now been resuscitated to the stage which is absolutely necessary for continued existence, such and so many are still wanting or still below the threshold of effective coöperation with other functions. For example, take the resuscitation of a liver cell. It has a certain structure and certain functions. A period of anæmia of a given duration puts a stop to these functions, alters, it may be, the structure of the cell in a definite way. The circulation is reëstablished, the functions of the cell return, perhaps in a definite order. It is again capable of forming glycogen from dextrose, but perhaps as yet incapable of synthesizing ammonia compounds to urea, or of separating from the blood the constituents of bile. It may perhaps be capable of forming glycogen, but not of performing the converse change of glycogen into dextrose or of producing the ferment by which the latter change is accomplished. Its structure, or what histologists denominate its structure (a more or less artificial picture perhaps) may be restored *pari passu* with the restoration of function, so that it might be possible to say, from a microscopic examination of the cell, at this moment it is capable of forming bile but incapable of forming urea, or the relation between the restoration of function and of structure may be a very complicated one, and extremely difficult to decipher. At what stage shall we say the resuscitation of a hepatic cell is completed? Again, it is very easy to reply, "When it does everything that the normal hepatic cell does in quantity and quality, and when its structure is the same." But again it is, at present, quite impossible, before resuscitation has been attempted, to predict whether, or how completely, it will occur. Take, for example, the resuscitation of a neurone. Undoubtedly the most important criteria of its recovery are that it should again be susceptible of being excited and of conducting the excitation. But we know that there are differences in the resuscitability of different parts of the neurone, the synapse, *e. g.*, being less easily resuscitated than the axone. And it is not easy to say at what precise point the threshold of resistance, whatever it may

be, at the synapse becomes so low that it can be considered normal.

When we take the case of an entire organ, the question is still more complicated. It is not only that the resuscitability of different cells of the organ may be different, that some of the hepatic cells, *e. g.*, having perhaps suffered definitive death while others are still resuscitable, just as certain erythrocytes in a given specimen of blood are always more resistant to the action of a given hæmolytic agent than others, but that the different tissues which compose an organ are, in general, of unequal resuscitability. If a certain proportion of the hepatic cells never recover any functions, and ultimately degenerate and disappear while others perhaps recover certain functions without recovering all, and the rest of the hepatic cells are completely restored, or if the vaso-motor and secretory nerves of the liver (assuming that the latter exist), or some of them, remain, after a given period of anæmia, incapable of resuscitation while the rest recover, by what practical criterion shall be decided the degree of resuscitation of an organ as a whole?

Where the resuscitation of a whole animal is considered, the question becomes still more difficult, for the completeness of the resuscitation of any given organ is inextricably dependent on the completeness of resuscitation of others.

We may, indeed, speak not only of the resuscitation of an organ or a function, but also of the resuscitation of a reaction, even a chemical reaction, in the animal body. And it may even be most philosophical to consider that effective resuscitation of an entire organ has been accomplished when certain fundamental and indispensable reactions are again taking place in proper sequence in time and proper distribution in space in the body. What, for example, has occurred when the respiratory center has once more begun to discharge itself but that certain reactions in abeyance for a time are once more running their normal course in the elements of the center and propagating themselves, or some other reactions which are normally linked with them, along the efferent axones? We say that the respiratory center and its efferent pathway have been resuscitated. What we actually observe is that the spontaneous discharge is again taking place. With what alterations in the so-called structure of its histological elements this discharge may be compatible, we do not at present know.

The possibility that in resuscitation certain chemical reactions of the liver cells may be separately restored has already been mentioned. Many other instances of the possible resuscitation of a reaction without the complete resuscitation of the organs themselves might be alluded to. For instance, it is known that curves representing the rate of oxygen consumption and carbon dioxide excretion in excised muscle subjected to artificial circulation at temperatures below that of the body diverge widely from each other, suggesting that these reactions are to a certain extent independent of each other. In resuscitation it is conceivable that the one might return while the other was still absent, or that the one might be fully resuscitated before the other. If muscle has a heat-producing mechanism separate from a mechanism by which it transforms chemical energy into mechanical work, the two mechanisms might be separately resuscitated. In the liver and other cells synthetic processes might be resuscitated at a different time from degenerative processes. The power of the cells of the intestinal villi of absorbing fatty acids and soaps might be resuscitated earlier or later than the power of synthesizing these substances to neutral fats. In the visual mechanism, the function of perceiving light might be resuscitated at a different time from the function of distinguishing colors. In the resuscitation of the lungs, the function of excreting carbon dioxide, so far as it does not depend on physical diffusion, might be resuscitated at a different time from the function of absorbing oxygen. This might perhaps be tested by clamping the division of the pumonary artery going to one lung for a time and then releasing it, the pulmonary artery to the other lung being now clamped permanently, and the gaseous exchange through the first lung being studied as it recovers from the consequences of the anæmia. In the case of the pancreas, the internal secretion might be resuscitated at a different time from the external secretion, and after a period of anæmia of the pancreas of proper length, an animal might recover one function and not the other—a possible means of testing the hypothesis that the internal secretion is formed by different histological elements (as the islets of Langerhans) from the external.

The differences in the relative resistances of the various organs

and tissues may be examined from another point of view. Eigenmann,[4] as the result of a long and extensive study of the degenerate eyes of the blind, cave-dwelling vertebrates of North America, concludes that the active structures of the eye, such as the retinal elements and the lens, degenerate sooner and more completely than the passive structures, such as the scleral cartilages. It has been shown,[5] also, that the passive structures, *e. g.*, the corneal cuticula, in the eye of a Cuban cave-shrimp are more resistant than the active structures of the dioptric apparatus, which is represented by the merest vestiges of this apparatus as it exists in the normal decapod eye. But of this dioptric apparatus, not all parts have suffered alike. The cone cells, which constitute the refractive apparatus in the decapod eye and cause the formation of an image, have almost totally disappeared, while the retinula cells, concerned with the perception of light and images, are present to about the number of one hundred. It was suggested that the difference in resistance shown by these two groups of cells might be connected in some way with the probable earlier phylogenetic development of the retinular cells. It is obvious that the stoppage of the circulation, resulting in the failure of the oxygen supply to the cells, or the total anæmia of an organ constitutes an adverse influence of marked severity. The question arises, therefore, as to whether or not we can trace a similar general relationship between the activity or passivity of a structure on the one hand, and the relative phylogenetic age of two closely related active structures or functions on the other, and the resistance of these structures or functions to such general necrotic processes as asphyxia or anæmia.

We may take as illustrative examples the following structures or functions. Bone may be regarded as a purely passive structure. So far as our experimental evidence goes, it seems to be almost unaffected by any agents which do not produce irrevocable loss of bodily functions. The tests for the functional activity of connective tissue in general are so ambiguous that we do not consider it profitable to discuss them here, but we have observed no symptoms in our animals which would lead us to believe that these tissues

[4] Eigenmann, The Mark Anniversary Volume, 1903, 167–204.
[5] Pike, *Biological Bulletin*, 1906, xii, 267.

were very much affected. It is commonly stated by surgeons that the epithelial cells of the skin will retain their vitality for one to two weeks, if left *in situ* and kept on ice, after amputation of a limb, and will grow when placed on a skin wound. We have never seen any case of permanent death of the integument of the head in animals subjected to cerebral anæmia. Integument, as well as bone, is a relatively passive structure, but both have other somewhat active structures intimately related to them—the erythroblasts in the bone, and the pilo-motor muscles and sometimes glands in the skin. We do not know to what extent these more active structures might suffer as compared with the inactive bones and skin.

In cerebral anæmia the muscles of the head region suffer less than the nervous system. A muscular contraction may be obtained by striking the muscle at a time when no reflex movement can be elicited in the same region.

Within the nervous system itself, there is a diversity of reactions to anæmia. The cortical centers succumb first and recover last. The respiratory and vaso-motor centers persist much longer and recover earlier, as we have pointed out before. Another example of different degrees of resistance in two closely related structures of about equal activity is found in the inhibitory and accelerator fibers of the heart-vagus nerve. It has previously been pointed out that after division of this nerve, the inhibitory fibers degenerate more rapidly than the accelerators.[6] We have shown that the inhibitory mechanism succumbs to anæmia sooner than the accelerator mechanism. Carlson,[7] in his study of the cardiac nerves in molluscs, found that some of the lower groups of the phylum have no inhibitory cardiac nerves, or at least none that were demonstrable, and suggests that the accelerator nerves to the heart arise earlier phylogenetically than the inhibitory.

There is little doubt that functionally, as well as morphologically, the cortical centers of the brain are of later phylogenetic origin than the centers concerned merely with the maintenance of life. We have shown that an animal may recover, so far as all the lower

[6] Schiff, *Arch. f. d. gesam. Physiol.*, 1878, xviii, 172; Arloing, *Arch. de physiol. norm. et path.*, 1896, Ser. V, viii, 75.

[7] Carlson, *Amer. Jour. of Physiol.*, 1905, xiv, 16.

nervous centers are concerned, but be almost totally lacking in intelligence.

So little attention has been devoted to phylogeny of function that, even if we possessed the necessary data as to the relative resistance of all the functions, we would still be unable to say whether or not they agreed in more than a very general way with the above hypothesis. But as the study of the ontology and phylogeny of structure has made of morphology the rational science which it is to-day, so we may look to the study of the ontogeny and phylogeny of function to give to physiology some of the generalizations of which it so much stands in need. Furthermore, it is completely in accord with modern views of evolution that a cell or an organism should acquire first those functions which are most necessary for life; and it is conceivable that these necessary functions should be the ones to succumb last to injurious influences in general, and the first to recover when the conditions again become favorable to life. To explain the nature of this resistance would again go far toward explaining the life processes in the cell.

PREVIOUS WORK ON RESUSCITATION.

We have reviewed the literature on some of the special phases of the subject in our previous papers, to which the reader is referred for a fuller discussion.

The idea of resuscitation is so old that it seems impossible to trace its origin. The Hebrew and other literatures of the ancient world contain many references to it, often under the guise of a miracle.

Among physiologists, Legallois[8] in 1812 is credited with the first statement of a belief in the possibility of the resuscitation of parts of an animal or even of a whole animal some time after death. But long before the time of Legallois, the experimental foundation of the subject had been laid by Stenson[9] and Swammerdam[10] in the same year. Stenson attributed the paralysis following ligation of the abdominal aorta to an affection of the peripheral nerves and muscles. Haller,[11] in the next century, held a similar view. Stannius,[12] about the middle of the last century, showed that the irritability of the spinal cord was diminished immediately after occlusion of the abdominal aorta. The credit of pointing

[8] Legallois, Oeuvres de Legallois, with notes by M. Pariset, Paris, 1830, i, 131.
[9] Stennonis, N., Elementorum myologiae specimen, etc., Florence, 1667.
[10] Swammerdam, J., Tractatus de respiratione, Ludwig, Batavia, 1667.
[11] Haller, Elementa physiologiae corporis humani, Lausanne, 1762, iv, 544.
[12] Stannius, *Arch. für physiol. Heilkunde,* 1852, xi, 1.

out the fact that the true cause of the paralysis lay in the anæmic changes produced in the spinal cord is almost universally given to Schiffer,[13] although Vulpian[14] had pointed out the same fact several years before. About two hundred years after Stenson described his experiment its true significance became apparent. The nerve cells of the spinal cord were more sensitive to anæmia than the muscles. Spronck,[15] some years later, showed that an animal might regain complete functional use of the spinal cord following total loss of such function during temporary anæmia.

Brown-Sequard[16] decapitated dogs and, after the head had failed to respond to electrical stimulation of the medulla, connected cannulas with the cerebral arteries. The injection of oxygenated blood caused apparently voluntary movements of the muscles of the face and eyes within two or three minutes. Cessation of the artificial circulation was followed by movements of the lower jaw, respiratory movements of the nostrils, and finally by dilation of the pupils as in ordinary death.

We have published in a separate paper[17] experiments showing that the activity of the decapitated head may be maintained for a considerable time after decapitation, by vascular anastomosis and the circulation of entire blood through it. Suitable nutritive conditions are sufficient to maintain a certain degree of activity of the decapitated head, and an unbroken connection with the spinal cord is unnecessary.

There had accumulated then, relatively early in the past century a considerable body of experimental evidence to the effect that certain parts of the nervous system might be restored more or less completely to a functional state after total loss of function if a proper circulation of oxygenated blood was begun in time. It early became apparent also that the central nervous system was more sensitive to anæmia than the muscles. It is self-evident that the length of time which may elapse after the death of an animal within which resuscitation of the whole animal is possible is determined by the resistance or viability of the part most susceptible to adverse influences or necrotic changes. This weakest part would appear to be the central nervous system. It is therefore of practical importance as well as of scientific interest to determine the period after which the central nervous system may be resuscitated *in situ*. Our experiments on this point, together with a partial review of the literature, have been published in a separate paper.[18] The time limit of total anæmia of the central nervous system after which complete resuscitation is possible is probably below twenty minutes. This is about the same limit set by Batelli[19] and by Mayer.[20]

[13] Schiffer, *Cent. f. d. med. Wiss.*, 1869, 579, 593.

[14] Vulpian, Leçons sur le physiologie générale et compareé du system nerveux, Paris, 1866, 451; *Gazette hebdomadaire de méd. et chirurgie*, 1861, viii, 365.

[15] Spronck, *Arch. de physiol., norm. et path.*, 1888, Ser. 4, i, 1.

[16] Brown-Sequard, *Jour. de la physiol. de l'homme et des animaux*, 1858, i, 117.

[17] Guthrie, Pike and Stewart, *loc. cit.*

[18] Stewart, Guthrie, Burns and Pike, *loc. cit.*

[19] Batelli, *Compt. rend. de l'Acad. des Sciences*, 1900, cxxx, 800; *Jour. de physiol. et path. gén.*, 1900, ii, 443.

[20] Mayer, *Med. cent.*, 1878, xvi, 579.

Although the early work on resuscitation dealt largely with the central nervous system, the impetus to recent work came largely from the experiments of Kuliabko[21] on the excised heart.

The resuscitation of the heart has been accomplished (1) by massage *in situ* and (2) by perfusion with some artificial or natural circulatory fluid.

Schiff,[22] correctly, and Hake,[23] erroneously, working independently, are credited with having first practiced direct massage of the heart. The method has come into clinical use with more or less indifferent success. The literature on resuscitation of the heart by direct massage up to the year 1902 is given by Boureau.[24] Prus[25] did an extensive series of experiments on the resuscitation of the heart by opening the thorax, employing Wehr's[26] technique, and massage of the heart. He reports freedom from infection and complete recovery in every way after long periods of stoppage of the heart. It should be pointed out, however, that Prus's means of determining the moment at which the heart stopped beating—the moment when the heart beat ceased to affect a mercury manometer is untrustworthy, and the long periods of time after which he reports complete resuscitation should therefore be accepted with considerable caution. The thorax was not opened until a certain time after the heart ceased to affect the manometer, and the exact time of cessation of the beat was not determined by inspection. In the resuscitation of a man two hours after death from suffocation, the heart must certainly have been beating a part of the time during which it was supposed to be stopped. As we have pointed out above, the period of total anæmia after which resuscitation of the central nervous system is possible is very much less than two hours.

The difference between clinical death and true death should be most closely borne in mind in this connection. There are cases recorded in the literature in which the heart has been observed to beat an incredible period after clinical death. Thus Rousseau[27] observed beats of a woman's heart at an autopsy twenty-nine hours after execution. Parrum[28] observed rhythmical contractions of the right auricle of a rabbit's heart fifteen hours after death. Vulpian[29] observed beats in a dog's heart ninety-three and one-half hours after death. Regnard and Loye,[30] in making an autopsy, observed beats of the heart lasting for about an hour after execution. The only certain way of testing whether or not the heart has stopped is by direct inspection. The heart doubtless continues to beat, although never so feebly, for sometimes minutes, perhaps hours, after it ceases

[21] Kuliabko, *loc. cit.*

[22] Schiff, *Arch. f. d. gesam. Physiol.*, 1882, xxviii, 200.

[23] Hake, *Practitioner*, 1874, xii, 241. Cited by Boureau, q. v.

[24] Boureau, *Revue de chirurgie*, 1902, xxvi, 526.

[25] Prus, *Wien. klin. Woch.*, 1900, xiii, 451, 482; *Arch. de méd. expér. et d'anat. path.*, 1901, xiii, 352.

[26] Wehr, *Arch. für. klin. Chirurgie*, 1899, lix, 949.

[27] Rousseau, *Compt. rend. de l'acad. des Sciences de Paris*, 1855, 11. Cited in Richet's Dictionary, iv, 312. q. v.

[28] Parrum, Bibliothek for Laeger, 1858, x, 46.

[29] Vulpian, *Compt. rend. des Séances et Mem. de la Soc. de biol.*, 1858, v, 1.

[30] Regnard and Loye, *Compt. rend. de la Soc. de biol.*, 1887, Ser. 8, iv, 433, 537.

to cause any movement of a mercurial manometer, and the feeble beat may maintain a circulation sufficient to sustain the life of the nervous tissues as well as of the other tissues. We incline to the belief that these facts, generally overlooked clinically and often experimentally, will explain many of the cases of resuscitation at long intervals after clinical death.

Kemp and Gardner[31] have reported a series of experiments on dogs in which they obtained resuscitation after death from chloroform. The method employed was (direct) massage of the heart and artificial respiration. Tracheotomy was not done but O'Dwyer's tubes were introduced through the larynx. The results were good.

Further summaries of the clinical aspects of cardiac massage have been given by Keen[32] and Lenormant.[33]

Since the appearance of Boureau's paper, Sencert[34] has reported a case in which he massaged the heart by introducing the hand through the abdominal wound made in the course of an operation and massaged the heart through the diaphragm. The recovery was complete.

Very recently, Green[35] has described two clinical cases of cardiac massage occurring in his own practice, and cites thirty-eight other cases from the literature. Green used the subdiaphragmatic method of massage, opening the abdomen and palpating the heart through the diaphragm by means of the fingers introduced through the abdominal wound. In one case the diaphragm and pericardium were incised and the heart palpated directly. The subsequent course in the first case was much like that which we have noticed in cats after temporary cerebral anæmia. There were variations in the pulse rate at varying intervals after starting the heart. Spasms soon appeared and continued more or less intermittently until death some twenty hours later. The temperature rose to an alarming degree.

Floresco[36] has reported a method for starting the quiescent heart by electrical stimulation. Two electrodes, insulated from each other, are passed downward along the carotid artery through a wound in the neck to the heart. Stimulation by induced currents caused the heart to beat after fifteen to forty minutes of stoppage (dogs). Here again we must exercise caution in accepting the results, since Floresco also took as the time of stoppage of the heart the moment when it ceased to affect a mercurial manometer. Concerning the possibility of starting the quiet heart by electrical stimulation we shall speak later on in the paper. The method, if practicable, would possess certain advantages over opening the thorax, such as avoidance of the ugly chest wound.

Resuscitation of the heart by perfusion of a suitable medium through the coronary vessels was also first practiced some years before the work of Kuliabko appeared.

Martin[37] is credited with the first perfusion of the mammalian heart through

[31] Kemp and Gardner, *Medical News,* 1903, lxxxiii, 184.

[32] Keen, *Therapeutic Gazette,* 1904, Ser. 3, xx, 217.

[33] Lenormant, *Revue de chirurgie,* 1906, xxxiii, 369.

[34] Sencert, *Jour. de méd. de Paris,* 1905, Ser. 2, lviii, 1080.

[35] Green, *Lancet,* 1906, ii, 1708.

[36] Floresco, *Jour. de physiol. et path. gén.,* 1905, vii, 785, 797.

[37] Martin, *Studies from the Biological Laboratory of the Johns Hopkins University,* 1890, iv, 275.

the coronary arteries. Langendorff[38] and Porter,[39] working independently, modified Martin's method by ligating all branches of the aorta except the coronaries, thus reducing the volume of blood necessary for a perfusion. The perfusion of the human heart was first done by Hedon and Gilis.[40] The earlier literature, together with a discussion of Martin's work is given by Magrath and Kennedy,[41] and by Porter. While it is of great scientific interest to know that a certain tissue may be roused to activity several days after death, yet, manifestly, it can be of no great practical importance in the resuscitation of an entire animal if it transcends the limit of the weakest link in the systemic chain. Herlitzka[42] has tried the effect of intra-venous and intra-arterial injections of Locke's fluid and adrenalin hydrochloride in the resuscitation of dogs after asphyxia. He avoided opening the thorax and introduced a sound through the carotid artery into the aorta. Injection of Locke's fluid to which a small amount of adrenalin had been added was accomplished through the sound, and the heart caused to beat by establishing the coronary circulation. Pressure in the right heart was relieved by withdrawing blood through an ordinary urethral catheter introduced into the right jugular vein. D'Halluin[43] repeated the experiments of Kuliabko, but failed to obtain the brilliant results claimed by the former investigator.

The work of Locke and others on the effect of solutions of inorganic salts upon the heart has been discussed in a separate paper.[44] The practical applications of the intra-venous or intra-arterial injections of the various salt solutions to the general problem of resuscitation will be discussed in a subsequent section.

A third line of work, subsequent to much of that on the nervous system but antedating most of the work on the heart, was a series of attempts to resuscitate an entire animal after accidental death. Goodwyn,[45] in 1788, wrote on the most effectual means of cure of submersion, strangulation and several kinds of noxious airs on living animals. Burgeois,[46] in 1829, and Kay,[47] in 1834, wrote on the physiology, pathology and treatment of asphyxia, including suspended animation in new-born children, and asphyxia from drowning, hanging, wounds of the chest, mechanical obstructions of the air passages, respiration of gases, death from cold, etc. Curry[48] wrote on the means to be employed for recovery from apparent death due to drowning, suffocation and other like causes. Few positive results were obtained.

A committee which was appointed (in 1862) to investigate the subject of suspended animation, reported to the Medico-chirurgical Society of London in 1862.[49]

[38] Langendorff, *Arch. f. d. gesam. Physiol.*, 1895, lxi, 261.

[39] Porter, *Amer. Jour. of Physiol.*, 1898, i, 511.

[40] Hedon and Gilis, *Compt. rend. de la Soc. de Biol.*, 1892, iv, 760.

[41] Magrath and Kennedy, *Jour. of Exper. Med.*, 1897, ii, 31.

[42] Herlitzka, *Arch. ital. de biol.*, 1905, xliv, 93.

[43] D'Halluin, Résurrection du coeur, Lille, 1904.

[44] Guthrie and Pike, *loc. cit.*

[45] Goodwyn, The Connection of Life with Respiration, etc., London, 1788.

[46] Burgeois, *Arch. gén. de méd.*, 1829, xx, 220, 508; xxi, 227.

[47] Kay, The Physiology, Pathology and Treatment of Asphyxia, etc., London, 1834.

[48] Curry, Observations on Apparent Death from Drowning, etc., London, 1815.

[49] *Medico-chirurgical Transactions*, 1862, xiv, 449.

Bailey[80] has reported a case of resuscitation after freezing. Some remarkable resuscitations have been reported by the editor of the *Lancet.*[81]

A fairly complete account of the condition of the visceral organs after the resuscitation of an entire animal is that given by Prus in his two papers.

THE EXPERIMENTAL RESULTS.

The General Conditions Affecting Resuscitation.—In attempting the resuscitation of an animal, we tacitly assume that no organ or system or constituent of the body which is absolutely essential to life, has suffered irreparable injury. It would be hopeless to attempt a permanent resuscitation after the blood had clotted in the head or great vessels or after putrefactive bacteria and other injurious organisms had entered into it in sufficient numbers to be fatal, or after the blood had been destroyed by chemicals or venom or had suffered such spontaneous changes as rendered it incapable of aiding in the resuscitation of the tissues or of nourishing them normally after they were resuscitated.

It is manifestly impossible to tell the exact moment of clotting of the blood within the heart unless the wall of one of the cavities is incised. After incision, the liberation of the kinase from the tissues of the heart will so hasten coagulation that we cannot take the result obtained as the true time of coagulation. The most trustworthy results will, therefore, be those in which the condition of the blood within the heart is stated exactly as it is found on incision of the ventricles. The results of our observations are given in Table I.

The dogs on which the observations were made had been used by students for experiments on the submaxillary gland, including stimulation of the chorda tympani nerve. In some cases a certain amount of dissection was done on the abdomen after death, such as removal of the stomach. The operative procedures had undoubtedly liberated a considerable amount of kinase from the tissues. Although the early occurrence of clots in our subjects is probably favored by the ante-mortem treatment of the animals, the interval during which the blood remained unclotted is greater than the period after cessation of the circulation after which the central

[80] Bailey, *Physician and Surgeon,* 1903, xxv, 502.
[81] Editorial, *Lancet,* 1904, i, 1005.

nervous system can be resuscitated. Intravascular clotting cannot practically be considered a barrier to resuscitation.

TABLE I.

Showing the condition of the blood in the cavities of the heart on incision at varying intervals after death. November, 1904.

Animal.	Manner of Death.	Time After Death at which Heart was Opened.	Condition of Blood in Heart when Incised.
1. Dog.	Asphyxia after ether.	100 min.	Moderately firm clots.
2. Dog.	Heart stopped from ether.	21 "	No clot.
3. Young dog.	Asphyxia after ether.	30 "	No clot.
4. " "	" " "	25 "	No clot.
5. Pup.	" " "	31 "	No clot.
6. Dog.	Haemorrhage and asphyxia.	115 "	Clot.
7. Dog.	Asphyxia after ether.	130 "	Clot.
8. Pup.	Chloroform.	60 "	Clot.
9. Pup.	"	120 "	Clot in right heart; none in left.
10. Dog.	"	36 "	Small clot in right ventricle; none in other cavities.
11. Young dog.	"	40 "	No clot.
12. Dog.	"	50 "	Moderate coagulation in right ventricle; clot in left ventricle.
13. Young dog.	"	49 "	Clot.
14. Dog.	Haemorrhage and asphyxia.	60 "	Clot.
15. Young dog.	Asphyxia after ether.	23 "	No clot.
16. Dog.	Anæsthesia.	176 "	Clot.
17. Dog.	Hæmorrhage and asphyxia.	153 "	Clot.
18. Young dog.	Asphyxia after ether.	40 "	Partial coagulation in right ventricle, but no clot in left ventricle.

Sollman[52] found that clots occurred in the heart cavities in two out of his three dogs within twenty minutes after death, a time somewhat shorter than that at which we found them. The presence of a clot in the ventricle is probably not, in itself, an absolute bar to the resuscitation of the heart. The left heart seldom contains much blood after death, and a clot found in its cavities would not, in general, completely fill them, and thus prevent the inflow of blood into these cavities. And again, the mere presence of clots, acting solely as foreign bodies in the ventricle, would present no great difficulty, as the introduction of sounds and catheters into the cavities of the heart for the measurement of endo-cardial pressure

[52] Sollman, *American Medicine*, 1904, viii, 455.

is a matter of common occurrence. Ante-mortem clots are occasionally found within the heart and do not seriously embarrass its action. In starting the heart by intra-arterial injections of fluid, the injected fluid, as will be pointed out later, does not necessarily enter the heart cavities at first, but goes into the coronary arteries and reaches the cavities of the heart only after passing through these vessels.

Permanent resuscitation of an animal after the formation of clots in the heart, although perhaps not impossible, is scarcely to be hoped for.

The Resuscitation of the Blood.

Since all that is necessary, under normal conditions, for the life of most tissues is a good circulation of normal blood, functional connection with the nervous system being apparently not indispensable, we are very early met by the questions: (1) What are the criteria of blood of sufficiently good quality for resuscitation of the organs? (2) How long does blood stagnating in the vessels after cessation of the circulation, or shed blood preserved under proper conditions, retain the necessary properties? (3) To what extent can blood which has lost some or all of these properties be itself resuscitated? The change which blood ordinarily undergoes in passing through the lungs, kidneys and other organs constitute in a sense a resuscitation of it, and are qualitatively perhaps the same as the change undergone by stagnant blood when it is again caused to circulate. But it is better to restrict the term to resuscitation of blood whose formed elements have suffered a decided loss of function, though not an irreparable one.

It is impossible to give any very definite answer to these questions. If bacteria have appeared in the blood, or if spontaneous laking has occurred, undoubtedly the blood is below the necessary standard, although even then the circulation of it through normal organs may restore it. Blood which has stood for many hours, or even days, in the ice chest may more or less successfully revive the isolated mammalian heart. The deleterious effect of waste products accumulated in the stagnating blood are not very conspicuous, since restoration after simple asphyxia is relatively easy.

It is known[53] that laked blood is harmful to the isolated heart on account of the liberated potassium salts. It is probable that the harmful effect in resuscitation of an entire animal would not be so great since elimination, or perhaps combination of the potassium salts with proteids, would occur. In any case, in the absence of bacteria or of hæmolytic agents like bile salt, laking of blood at ordinary, and even at body, temperature takes place only after a long interval. An example of the spontaneous aseptic laking of dog's blood is given in the following protocol.

February 13, 1905. Blood was run through a sterile cannula from the carotid artery of a dog into a number of sterile glass tubes which were then sealed in the blowpipe flame.

March 15, 1905. All the specimens are now well laked. There is still plenty of clot in all the tubes. The spectrum was that of reduced hæmoglobin. The color was dark purple.

September 1, 1907. Between this and the last date the tubes were repeatedly examined, without revealing any change. Still much clot, the autolytic processes having ceased to affect the fibrin.

The spectrum is still that of reduced hæmoglobin, except in a tube which was slightly broken at one end. Here the color is brown and the spectrum shows a strong band in the red in the position of the methæmoglobin band. One of the other tubes was shown to be free from bacteria.

As the necessary condition for the resuscitation of the solid tissues is a supply of good blood under proper pressure, the necessary condition of the resuscitation of blood is a good circulation of it through a body whose tissues are in a sufficiently normal condition, or, at any rate, through a certain number of indispensable tissues. Since, in an animal which is being resuscitated under ordinary conditions, neither the blood nor the tissues are normal, the question presents itself whether its own previously stagnant blood is the best circulating medium, or whether it might not be improved by the addition of artificial liquids or of blood from a normal animal of the same species. Crile, for instance, has recently revived the operation of transfusion for the treatment of such conditions as coal-gas poisoning. Since here the serious factor is that the hæmoglobin has ceased to act as an oxygen carrier, it is possible that the introduction of a certain amount of hæmoglobin in salt solution, a simpler proceeding than transfusion, might be efficacious. But

[53] Langendorff, *Arch. f. d. gesam. Physiol.*, 1903, xcix, 30.

hæmoglobin from an animal could only be used if it were demonstrated that it did not exert an injurious action from the formation of anti-bodies, for instance.

The time when the blood loses its bactericidal power so that micro-organisms may pass into it from the alimentary canal is not devoid of interest in this connection. It is not probable that the epithelium and other defenses of the organism should prove to be the weakest link in the chain and break down within a period less than that after which resuscitation of the central nervous system is possible, although it has been shown that the epithelial cells of the intestine lose their power of absorbing water from serum placed in the lumen for a long time after they have been subjected to anæmia for a period of fifteen to thirty minutes.[54] Nor is it probable that the blood would lose its bactericidal power toward the ordinary bacterial parasites of the colon within the same time. Longcope[55] found that the blood exerts some action against the *Bacillus typhosus* and *Bacillus coli.* In four to six hours after death some of the common organisms of the intestines are to be found in the neighboring tissues unless the cadaver has been put into the refrigerator immediately after death. This period, however, greatly exceeds that within which we may hope for a resuscitation of the central nervous system, and is, therefore, not a consideration of great practical importance. In addition to the loss of its biological properties—the hæmolytic and bactericidal powers—the serum suffers certain physico-chemical changes,[56] even when sealed in sterile tubes, such as changes in the depression of the freezing point, and changes in electrical conductivity, viscosity and coagulation by heat. The significance of these changes as regards the use of the blood by the tissues is not yet apparent, and none of them would occur within the time elapsing after stoppage of the circulation after which resuscitation of the central nervous system could be hoped for.

Cobra and crotalus venom, as is well known, have a violent hæmolytic action, and death results from the changes in the blood. No means of restoring this laked blood to its former physiological

[54] Reid, *Philosophical Transactions of the Royal Society,* B, 1900, cxcii, 240.
[55] Longcope, *University of Penn. Med. Bull.,* 1902, xv, 331.
[56] Buglia, *Archivio di Fisiologia,* 1906, iv, 56.

condition is known, and a suitable fluid, other than blood itself, which might be used for replacing the damaged blood and sustaining the life of the animal until it could form new blood has not been found. Whether transfusion of blood from another animal, allowing the laked blood to escape, would result in recovery is, of course, an open question.

The attempt to resuscitate the blood by transfusion has, of late, been made in some cases of anæmia and in the extreme cachexia of typhoid fever, with only indifferent success. If it be true that a sufficient supply of normal blood is all that is necessary for the life of the tissues, it would seem that, in cases where transfusion was not followed by recovery, the seat of the trouble lay in the cells; that the cells at the time when the transfusion was made, had already lost some of the functions necessary to life. So far as these functions are concerned, the cells might, therefore, be regarded as dead beyond the possibility of resuscitation, and total death would be a matter of a short time only.

The effect of defibrinated blood in maintaining the activity of the heart and some of the secretions is shown in the accompanying protocol. The results are not as good as when fresh blood is circulated through these organs, as we have good experimental reasons for thinking that the cells would have recovered if they had been supplied with fresh blood. The reason for this difference is unknown to us, but we believe that the experiments point toward the conclusion that blood soon undergoes changes which affect even its nutritive, as well as its bactericidal properties.

Experiment of March 1, 1905.—Pup, etherized, was kept in hot box.

11:23 A. M. Tied right subclavian artery and vein. Artificial respiration.

11:26. Tied aorta and put cannula in central end.

11:28. Tied inferior vena cava, after elevating hind end and bandaging abdomen.

11:38. Put cannula in aorta, toward heart, running it up into the auricle. Corneal reflex present.

11:39. Tied heart in auriculo-ventricular groove, omitting great veins, and immediately began artificial circulation with dog's defibrinated blood, obtained the day before and kept in the ice box.

11:48:45. Exposed vago-sympathetic trunks in neck without ligating or dividing them.

12:10 P. M. Auricles and ventricles still beating, but not with same rhythm (right auricle 20 in 17 seconds; right ventricle 20 in 45 seconds).

12:16. Added Locke's fluid to some of the blood (equal volumes) and began injecting it.

12:30. Stimulated right vago-sympathetic. Some slowing of right auricle (left not observed) and distinct after acceleration.

12:33. Put a little ether into the mouth. It seems to excite little secretion, but perhaps some was excited.

12:40. Œsophagus about two inches above auricle is beating, with almost same rhythm as auricle. Not very strong beats, but regular, except that occasionally there is a series of two or three beats more rapid than the rest. This more rapid series is not seen in the auricle.

12:51. Ligature was taken off of heart.

12:51:15. Ventricle began beating.

1:10. Superior vena cava and right auricle beating well. Closing outflow tube in inferior vena cava stops auricular beats at once, but does not affect superior cava beats. Verified many times. A very slight pressure in right auricle is sufficient to stop beats. Stimulated right vago-sympathetic. No stoppage of superior cava or auricular beats, but rather an acceleration.

1:22. Stimulated left vago-sympathetic. Still slight opening of left eye, and apparent retraction of nictitating membrane, but the latter may be due to bulging of the eye. (Both eyes have had marked increase of intra-ocular pressure, progressing since the time when the corneal reflexes disappeared (11:48:45) and have been permanently bulged out to an increasing degree.) Auricle is now beating much faster than the superior vena cava, which is beating strongly. Closing outflow tube stops auricle, but does not affect cava. After releasing tube, auricle beats more strongly than before. Some secretion is running from nose. Stimulated left vago-sympathetic. No effect on heart or superior cava.

1:30. Perhaps some increase in lachrymal secretion.

1:32. No salivary secretion, as shown by drying mouth previously.

1:36. Injected pilocarpine.

1:39. Stimulated left vago-sympathetic. Good retraction of nictitating membrane, without any opening of the eye. Some increase of lachrymal secretion in right eye.

1:53. Increased pressure of injection of blood to quicken flow.

1:57. Œsophagus active to direct stimulation.

1:58. Circulation is twice as rapid as before. Good lachrymal secretion, especially of right eye.

2:04. Stimulated right vago-sympathetic. Retraction of nictitating membrane and opening of eye. Now closed inflow tube, and left everything *in situ*.

3:03. Œsophagus and tongue still respond well to stimulation. Stimulated both vago-sympathetics. No effect on eyes. Skeletal muscles of shoulder excitable to direct, but not to indirect stimulation.

3:17. Started circulation. Right auricle soon beating well.

3:28. Stimulated vago-sympathetics. No effect on eyes. Right auricle beating well. Any pressure on outflow tube stops it. When pressure is relieved it beats more strongly than before. Superior vena cava is not beating, nor left auricle. Much secretion flowing from nose, tinged with blood pigment.

3:42. Tongue still excitable to direct stimulation. (Did not try indirect.) Nictitating membranes are getting œdematous and bulge out. Some liquid is

gathering in mouth and throat, free from hæmoglobin. Tongue getting œdematous.

3:56. Stimulated right vago-sympathetic. No effect on eye. Liquid is coming freely from eye. Liquid also collecting in mouth. Gums wet.

4:08. Stimulated vago-sympathetics. No effect on eyes. Incised cornea in one eye to let the aqueous humor escape. It is somewhat tinged with blood. Vitreous humor also allowed to escape. Some blood seen upon or in it. Stimulation of the vago-sympathetic does not affect the eye any more than it did before. (Pressure was relieved in eye as it was thought possible that it might prevent mechanically any change in the pupil.)

4:20. Tongue still excitable, although swollen. On incision, œdematous fluid escapes from it. Œsophagus excitable. Large hemorrhage in its walls. Shoulder muscles contract on direct stimulation, but not on stimulation of nerves in axilla. Same for pectoral muscles. Hind leg muscles inexcitable to direct stimulation. Stimulation of sciatic nerve produces no effect. Experiment stopped.

Another experiment on the artificial circulation of defibrinated blood, to which peptone had been added, through the head end of an animal is of interest in connection with the formation of anti-coagulant bodies in this region. We give the protocol.

Experiment of April 8, 1905.—Small bitch. Ether. Hot box. Artificial respiration. Put a cannula in upper end of thoracic aorta and one in inferior cava. Circulated defibrinated dog's blood (obtained eighteen hours before and kept on ice) from bottle through head end.

11:22 A. M. The circulation was poor from this time. Did not tie off heart, but it was scarcely beating.

11:31:45. Began injection. Pupils not dilated. No eye reflexes, nor any other reflexes.

11:35. Twitching of neck muscles in the wound which was going on when the artificial circulation was begun is stronger. Pupils same as before. Tongue has a poor circulation. Artificial respiration was now stopped as heart was not beating.

11:43. Temperature in thorax 33.5° C. The blood, although stirred as it came out of the venous cannula and always strained before being reinjected, has clotted in the wound and no doubt in the blood vessels also.

11:48:30. Interrupted circulation to get rid of clot.

11:56:30. Started circulation again. Twitching of the neck muscles had all disappeared in the interval. No secretion of saliva.

12:05 P. M. Circulation is now good for the first time.

12:07. Tongue is excitable to direct stimulation but needs strong shocks.

12:10. Drew off a tube of blood (1) from the venous cannula.

12:24. Added about ten grams Witte's peptone in 0.9 per cent. sodium chloride to the blood in the artificial circulation.

12:35. Tongue still responds to strong stimulation.

12:38. Stimulated the vago-sympathetic. No effect on eyes. Direct stimulation of neck muscles causes strong contraction. Similarly with the œsophagus.

12:39. Took off another specimen of blood (2) from venous cannula.

12:51. Stimulation of the accelerantes (annulus of Vieussens and inferior cervical ganglion). No effect on heart. Heart had long since stopped. Direct stimulation of the heart causes a beat each time. Massage causes a series of good beats. Heart soon stopped but massage again started good beats, and so on.

12:55. Drew off another specimen of blood (3) from venous cannula.

After twenty-one hours at room temperature no coagulation appeared in (2) or (3). Slight coagulation in (1). Object of the experiment was to see whether the anti-coagulant action of peptone is developed in this limited circulation, but the blood had obviously been rendered almost incoagulable before the peptone was injected.

The Substitution of Other Fluids for Blood.—The value of the various inorganic solutions as media for maintaining the activity of the mammalian brain and heart, respectively, have been considered in separate papers.[57] None of these fluids will sustain the activity of the brain or higher nerve centers for any length of time, and all are ineffectual in maintaining the normal activity of the heart for more than a brief period. Certain points in the action of these fluids upon other organs or systems, and particularly their efficiency as compared with blood will be presented here.

The effect of these solutions in maintaining the reflexes is shown in the following protocol. It will be noted that the results are less satisfactory than when defibrinated blood is used.

Experiment of February 27, 1905.—Large male cat. Ether. Kept in hot box to prevent cooling.

2:05 P. M. Put cannula in central end of right carotid artery and bled animal.

2:10. Finished bleeding. Drew off about 50 c.c. of blood. Defibrinated it and mixed one part of blood with eight parts of Locke's solution. Maintained artificial respiration.

2:38:30. Ligated aorta.

2:42:30. Ligated inferior vena cava, after elevating hind end, squeezing and bandaging abdomen. The heart, which after ligation of the aorta had been beating rather feebly, was at once strengthened. Ligated right and left subclavian veins and arteries.

2:58. Slight light reflex in left pupil. Fair corneal reflex.

2:59. Interrupted circulation by ligatures under innominate and left subclavian arteries. Corneal reflex disappeared in 20 seconds, lid reflex in 45 seconds.

3:01:15. Still strong spontaneous respiratory movements.

3:01:45. No longer any spontaneous respiratory movements.

3:02. Pupils widely dilated. No light reflex.

3:06. Removed ligatures from innominate and left subclavian arteries.

3:08. Strong spontaneous rhythmical respiratory movements. Began injec-

[57] Guthrie, Pike and Stewart, *loc. cit.* Guthrie and Pike, *loc. cit.*

tion of blood mixture into central end of aorta, but injection failed because of a clot in the aortic cannula; removed clot.

3:10:30. Began injection of blood mixture.

3:11. Heart stopped. Kept up circulation by heart massage.

3:15. Stopped injection.

3:20. No return of eye reflexes as yet.

3:28. It was now seen that the left carotid had been included in ligature about left subclavian, and no blood had been passing through it since 2:51 P. M.

3:29. Stopped massage of heart.

3:31. No light reflex. Released left carotid from ligature.

3:32. Began massage of heart again, and injection of blood mixture.

3:36. Stimulated left vago-sympathetic; perhaps slight opening of eye.

3:38. Stimulated left vago-sympathetic. Undoubtedly some openeing of eye.

3:40. Stimulated vago-sympathetic. Some opening of eye.

3:45. Started massage of heart after an interval of four minutes. Added adrenalin to blood mixture and began injecting it.

3:48. Stimulated left vago-sympathetic. No effect on eye. It is now much easier to maintain good blood pressure than before the adrenalin was added. Very slight and slow massage of the heart suffices. The heart even gives feeble beats, although it has been quite motionless before. Beats seem to be synchronous with artificial respiration.

3:52. Stimulated left vago-sympathetic. No change in eye. Œsophagus contracts strongly during stimulation. (The nerve was tied but not divided, and was stimulated central to the ligature.) Neck muscles now excitable.

4:08. Stimulation of vago-sympathetics gives no effect on eye on either side. Direct stimulation of eyelids causes them to contract strongly. Good secretion of saliva seems to be going on. Tongue excitable. No trace of rigor in head end of animal. Stimulation of axillary nerves on left side causes strong contractions of muscles of fore-limb. Did not try on right side.

4:15. Stimulated cortex cerebri on left side. No movements of fore-limbs nor any eye movements. No change in pupils. Pushed electrodes down one and one-half centimeters into the brain (to strike fibers of corona radiata). No effect of stimulation. Even strongest currents produced no effects. Stimulation of axillary nerves is now ineffective on both right and left sides.

Artificial Respiration.—Another necessary condition for the life of the tissues is a sufficient supply of oxygen. Inasmuch as an animal apparently dead is incapable of oxygenating its own blood, some form of artificial respiration becomes necessary.

Artificial respiration by manipulation of the thorax has proved of little value in any serious accident with the anæsthetic. In general, if ether is the anæsthetic used, one may usually resuscitate an animal, a dog, for example, if the thoracic manipulation is begun immediately after the last gasp but before the heart has ceased to beat, and continued at about the normal respiratory rate until spontaneous respiration is again established. Owing to the laxity of

the pectoral muscles, movement of the fore-legs, alternately drawing them up above the head and pressing them downwards against the ribs is useless in a serious case. Sudden, firm pressure applied to the sternum and to the ribs on either side so as to bend the ribs and costal cartilages well in, and a sudden release has proved more efficient in our experiments. An objection to this method is that it often results in injury to the lungs from too violent compression of the thorax. Rhythmical inflation of the lungs by means of a pump or by compressed air is the most effective method we have tried. Care should be taken not to inflate the lungs too strongly, as the increased resistance caused thereby in the pulmonary vessels may be sufficient to stop a feebly beating heart, or rupture of the pulmonary alveoli may occur. Our technique for artificial respiration was given in our first paper[58] on resuscitation, and we will not, therefore, go into further details here.

In certain experiments on the cardiac nerves in which it was necessary to keep up artificial respiration because of opening the thoracic cavity, we observed that ether might be administered long after the respiratory movements had ceased without seriously affecting the heart. The tissues became cyanotic and the blood pressure fell very low, but on discontinuing the use of the anæsthetic, the heart recovered as soon as the blood became well oxygenated.

The possibility of affecting the heart directly, or even of starting beats, by artificial respiration is shown in the protocol, just quoted, of the experiment of February 27, 1905, in which the beats were apparently synchronous with the artificial inflation of the lungs. We have often found that artificial respiration alone is sufficient to restore to activity a heart almost stopped from ether anæsthesia, and particularly from asphyxia, a considerable time after respiratory movements have ceased. The efficiency of artificial respiration alone in starting a heart almost stopped from asphyxia is still more strikingly shown in the following condensed protocol.

Experiment of March 6, 1905. Young male cat, about two-thirds grown. Ether.

10:24 A. M. Occluded head arteries. Eye reflexes and respiratory movements gone in 20 seconds.

[58] *Jour. of Exper. Med., loc. cit.*, p. 292.

10:25:20. A gasping movement.

10:26:40. Respiratory gasp. These gasps have occurred at intervals (8 in 1 minute and 40 seconds).

10:27:20. Released head arteries. Put a glass tube into the larynx.

10:29. Began artificial respiration. No heart beat perceptible at this time, so that really the interruption of the circulation was total for three minutes and twenty seconds, and must have been almost total for one minute and forty seconds more. Heart soon began to beat well.

10:34:10. No reflexes (corneal or light). No movements of any kind.

10:38:35. A gasp. Pupils, which were widely dilated, are getting narrower. Subsequent recovery rapid.

The Resuscitation of the Heart.

We have studied the resuscitation of the heart more extensively than any other organ or system, with the exception of the central nervous system, and as it is the *sine qua non* in the resuscitation of an entire animal, we will consider it first of all. As it has been found that certain influences and modes of death are more injurious to the heart than others, thus rendering resuscitation of the animal more difficult, we shall consider the various modes of death separately.

The Criteria of Heart Stoppage.—We have already mentioned, in the survey of previous work, that the cessation of the oscillations of a mercurial manometer, or the failure of the carotid or other pulse, is an uncertain means of telling whethor or not the heart has stopped. In our experiments the heart has been exposed freely so that the base was easily visible, but mechanical stimulation of the organ was carefully avoided. The length of time elapsing between the cessation of the external pulse and the complete stoppage of the heart is given under the various modes of causing the death of the animals.

As a rule, the stoppage of the heart is accompanied by a convulsive movement or other outward manifestation on the part of the animal, but that heart stoppage may occur without any such outward sign is shown by the two experiments cited below.

Experiment of May 27, 1905.—Adult male cat. Tube in larynx.

9:09 A. M. Head arteries occluded for 45 minutes. Reestablishment of cerebral circulation. Artificial respiration when necessary.

8:07 P. M. Rectal temperature 38.2° C.

8:21. Cornea rather slack. Pupils about half maximum diameter.

8:28. Pulse 209 in the minute.

9:06. Stimulated the left brachial plexus through skin on that side. Strong tetanus of left forelimb. Pulse 187.

9:28. Stimulated right vagus in continuity. Stops heart at once, and causes maximum dilation of the pupil, bulging of the eye and retraction of the nictitating membrane. Repeated with same result. Muscles of the neck are excitable to direct stimulation. Rectal temperature 38° C.

9:47. Right eye still bulging widely, pupil maximum. Nictitating membrane retracted as before. Pupils and membrane have not come back to normal since stimulation of vagus. (Suggesting that contraction of the pupil to half maximum diameter, previously observed, might not have been due to oculo-motor tone.) Left eye same as before.

9:56. Right eye now bulging less; pupil diminished somewhat, but not to what it was before the stimulation. Nictitating membrane partly protruded, but not as much as before stimulation.

10:15. Heart cannot be felt. Opened chest and found it not beating, not even an auricle moving. There was no convulsion, nor movement of any kind to indicate at what time it stopped. Stimulation of phrenic nerve still causes contraction of the diaphragm.

It might be supposed that, on account of its great length, this was an unusual experiment, and that a similar result would not be obtained under other conditions where the spinal centers were not so profoundly exhausted. That this is not the case is shown by the following experiment, in which heart stoppage occurred within a relatively short time after the release of the head arteries. Both experiments go to show the unreliability of outward signs as an indication of the actual condition of the heart.

Experiment of May 29, 1905.—Adult male cat. Ether. Tube in larynx. Artificial respiration.

2:50 P. M. Occluded head arteries, in usual way.

3:31. Released head arteries.

3:52. Pulse 150. Corneal tension increased. Pupil a little less than maximum.

3:59. Rectal temperature 35° C. Pulse 162.

4:20. Noticed that the heart had stopped. No muscular contractions announced the time at which this occurred. Tried massage through chest, but although the heart was felt slipping up and down between the fingers, it could not be started. The pupils became narrower however. Opened chest, and found the heart quite motionless.

After Death from Asphyxia, Drowning, Etc.—We have studied the resuscitation of this form of death more in detail than other forms, and we will therefore consider it in detail, reserving for subsequent sections only the more important differences, the technique

employed and the results obtained in resuscitation after other forms of death. In general, we may say that resuscitation appears to be easier after asphyxiation than after other modes of death. Hemorrhage and anæsthesia tend to make resuscitation more difficult than after asphyxia alone. In order of increasing difficulty of resuscitation we would rank the forms of death as (1) asphyxia, (2) anæsthesia, (3) hemorrhage, (4) electrocution, by the action of induced currents upon the heart.

In nearly all cases the etherized animals were asphyxiated by clamping the trachea. The blood pressure falls to zero plus the residual pressure, within three to five minutes after the trachea is closed, and no oscillation can be detected in a mercury manometer connected with the carotid. On direct inspection, the auricles, and more particularly the right auricle, may be seen to beat for five to ten minutes or even longer.

In two experiments a piece of sheet rubber was tied firmly over the nose and mouth of the unanæsthetized animal.

Drowning was accomplished by immersing the head of the anæsthetized animal in water.

Hemorrhage was sometimes allowed to occur in case of asphyxia, and invariably rendered resuscitation more difficult than after asphyxia alone.

Resuscitation of the Heart by Direct or Intra-thoracic Massage.—This form of massage, as shown by the increase of blood pressure, and the relative certainty with which the heart is started to beat is more efficient than other forms. The heart may be started ten or fifteen minutes or even longer after cessation of the external pulse. When the aorta is occluded so as to confine the circulating blood to the anterior part of the animal, we have obtained resuscitation of cats as late as forty-four minutes after cessation of the external pulse. Prus reports a case of resuscitation of the heart in a dog after direct massage had been employed for two hours.

Doubtless this form of massage also facilitates the removal of waste products from the heart as it does from the skeletal muscles.

The prompt recovery of the heart as a result of direct massage after clamping the aorta is shown in the following experiment.

Experiment of March 18, 1905, Direct Massage.—Cat. Ether.

11:28 A. M. Occluded head arteries.

11:40:45. Released head arteries. Gave ether, as animal was apparently conscious. Soon after this animal stopped breathing. Heart could not be felt. There was certainly no circulation in the brain for from 15 to 20 minutes, and no respiration, in addition to partial anæmia during occlusion. Opened chest, clamped aorta and started artificial respiration.

12:05. Heart started again by massage. Artificial respiration kept up. Heart massage continued at intervals when heart needed it, compression of ventricles being made at the moment when they were felt to be beginning their contraction. Soon massage was unnecessary.

12:20. Pupils still at maximum dilation, eyes wide open; no light reflex. Heart beating well.

12:25. Tears are being secreted, and pupils are somewhat less. Left room.

12:43. On returning cat found gasping. Pupils distinctly less.

A modified form of intra-thoracic massage consisted in producing rhythmical changes of pressure within the pericardial sac. A wide cannula was tied into a small opening in the parietal pericardium, connected with a pressure bottle or a rubber syringe, the whole being filled with salt solution or warm olive oil. By rhythmically raising and lowering the bottle, or compressing the syringe, the intra-pericardial and therefore the endo-cardial, pressure was alternately increased and diminished. It was supposed that this would be less injurious to the heart than long continued massage with the fingers. The results were unsatisfactory.

Efficient as the method of manual massage is, there are obviously two objections: the large chest wound, with the attendant risk of infection, and the danger of actual mechanical injury to the heart. Many attempts have therefore been made to start the heart without recourse to opening the thoracic cavity.

Extra-thoracic Massage.—Rhythmical compression of the thorax over the heart by means of the hands has given fairly good results in certain stages of the heart stoppage; the time when such massage is effective is, however, much too limited to make the method a sure one. Rhythmical compression of the thorax is efficient up to from three to five minutes after the cessation of the external pulse, but it is probable that in every case of successful resuscitation by this method, the heart has not entirely ceased beating. Where we have been sure that the heart has stopped entirely, although for the briefest interval, extra-thoracic massage alone has proved useless.

The degree of efficiency of extra-thoracic massage in favorable cases appears from the subjoined protocol.

Experiment of May 21, 1905.—Cat. Ether. Tube in larynx.

11:59 A. M. Occluded head arteries.

12:40:50. Natural respiration stopped. No gasps after this.

12:05:15. Released head arteries. Heart beating well.

12:08:20. Gasps began.

12:14. No light, lid, corneal or ear reflex. Tube slipped out of larynx. Respiration interrupted for three minutes. At end of that time the pupils were at maximum dilation. Massaged chest. No heart beat can be felt. Cat seems dead.

12:25:20. Heart now felt for the first time. Artificial respiration interrupted until 12:26.

12:26. Artificial respiration was again started. Massage of chest continued uninterruptedly.

12:28. Heart now felt. Beating well.

Often a faithful trial of extra-thoracic massage has failed to start the heart. In nearly all of these cases direct massage has afterward proved effective. We cite one experiment.

Experiment of March 23, 1905.—Large adult male cat. Ether. Tube in larynx. Paralysis of respiratory center from ether. Artificial respiration started without result.

2:18. Heart could not be felt at all. No pulse in carotid (exposed in neck wound). Pupils widely dilated. All reflexes gone. Kept up vigorous massage of the chest for twenty-two minutes, elevating hind end of animal and trying to compress abdominal aorta, with the hand, through the abdominal wall. (Compression tried after massage had been continued for ten minutes without result.) No return of the heart beat occurred. No pulse whatever visible in the carotid. Artificial respiration had been kept up all the time.

2:40. Opened chest, clamped aorta and massaged heart directly. It soon started, but not very strongly. After a little time, the ventricles began to fibrillate while the auricles still beat fairly well.

2:42:30. Pupils have become somewhat smaller.

2:45:30. Heart is beating fairly well, although not so well as in most of the previous similar experiments. The fibrillary contractions are gone. (The abdomen had been opened at the same time that the aorta was clamped, and the intestines, kidneys and other viscera freely manipulated.)

3:08. Heart which had become very poor (*e. g.*, the ventricles beating only once for two auricular beats) was restored by massage and is now beating well.

3:31. First movement seen, viz., twitching of the skin over right shoulder. The twitching is superficial (platysma myoides) as shown by cutting through the muscle and exposing the deeper ones. Later on, the deeper ones began to twitch also.

Rhythmical compression of the thorax of a large dog at a necessary rate for resuscitation is exceedingly laborious and often can

not be kept up for a sufficiently long time. An attempt was made to devise a machine for this purpose. In the development and testing of this Professor J. L. Kessler was associated. Fig. 1 shows the arrangement. Fair results were obtained with this machine, although not so good as results of manual compression, so far as increase in arterial pressure produced before the heart started is concerned. The anticipated advantage of an indefinitely long massage was realized to some extent.

The relative efficiency of manual massage, rhythmical distension of the pericardial sac and mechanical massage will be made sufficiently clear from the following condensed protocol.

Experiment of February 6, 1904.—Young dog weighing ten kilograms. Injection subcutaneously of 10 c.c. of 0.2 per cent. solution of morphia; A. C. E. mixture. Respiration became rapid and shallow, and ceased within about twenty minutes after giving the A. C. E. mixture, and about five minutes after inserting the tracheal cannula. Heart massaged through the chest wall. Respiratory movements soon returned. Stopped massage. Heart beats and respiration soon stopped. No eye reflexes. This procedure was repeated twice.

Now tried mechanical massage with machine. Results were similar to the preceding.

Now opened chest and massaged heart directly. Good carotid pulse obtained. Elevated hind part of animal and connected the carotid with a manometer. A blood pressure of 20 mm. resulted without massage; this pressure was doubled by direct massage, but no pressure above 40 mm. could be obtained by rapid massage. Clamped aorta. Blood pressure now somewhat higher.

Stimulated accelerators on left side with moderately strong tetanizing current from an inductorium. Slow rhythmical beats occurred near the apex of the ventricles lasting about one to two minutes. Close inspection showed no beats and no fibrillary contractions prior to this.

Tried massage with machine. The effect upon blood pressure and pulse was not so good as that obtained by manual massage.

Tied cannula in the pericardium and connected with a syringe filled with water. Rhythmic pressure on the syringe produced only low blood pressure and poor pulse.

Intra-venous and Intra-arterial Injections.—Nothing is more disappointing than the deportment of the heart following injection of 0.9 per cent. sodium chloride or Locke's solution into the carotid artery or jugular vein. The heart remains as immobile as before, or fibrillates a little before going into rigor; the injected fluid slowly but surely distends the right auricle if injected into a vein, and escapes into the peritoneal cavity and also into the lungs, giving rise to pulmonary œdema which would, in itself, prove fatal. But

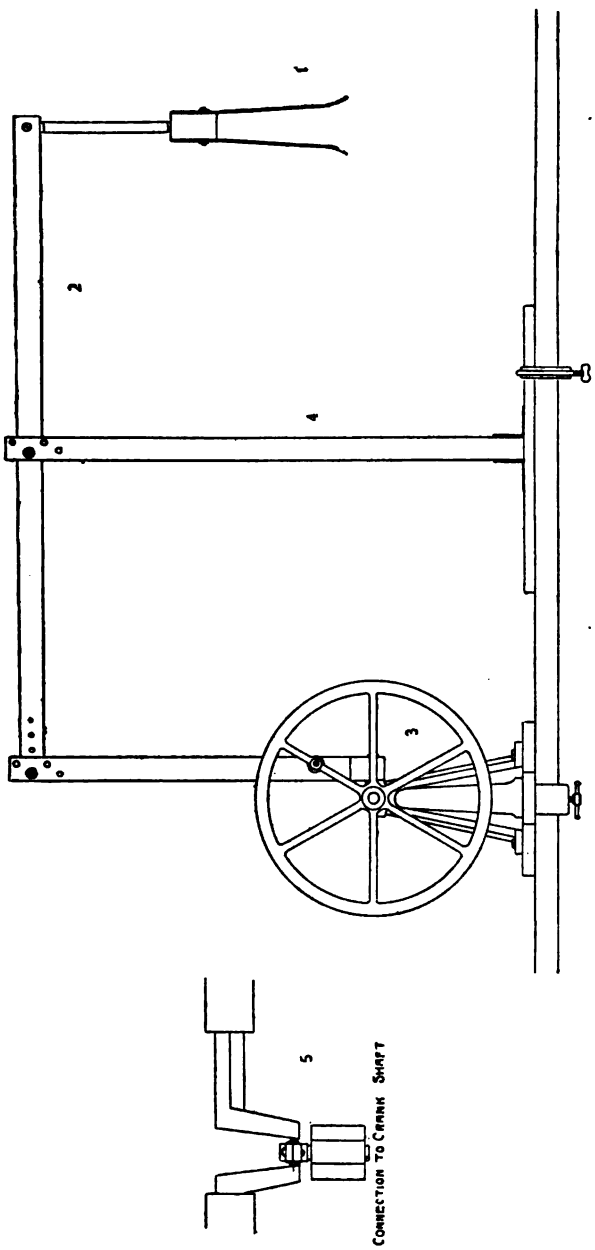

Fig. 1. Showing the arrangement of Professor Kessler's machine. Two sheets of heavy spring brass (1) are bolted to a block and attached to an oscillating beam (2). This, in turn, is attached to a crank shaft (3) revolved by a wheel. A bolt passes through the upright (4) and the beam (2) at their junction. The details of the attachment to the crank shaft are shown in (5). By revolving the wheel, the spring brass sheets are alternately raised and lowered. The animal is placed on a board, the chest covered with towels to protect it from the bruising, and put beneath the brass plates. The rate of compression can be regulated by regulating the speed of revolution of the wheel, and holes in the uprights and in the beam permit of an adjustment of the stroke.

quite apart from the œdema or the occasional rigor produced in the ventricles, the presence in the vessels of such a great quantity of fluid as is usually injected would be likely to overtax the heart, even if it began to beat. Indiscriminate and unconfined injections of fluid are, therefore, worse than useless. This agrees, in general, with Crile's experience.

The suddenness of the onset, and the severity of this œdema, even before the death of the animal, are shown in the following protocol of a typical experiment, in which also some data on the persistence of the reflexes are incorporated, in order to show the effect of artificial fluids in maintaining the activity of the higher centers. In an animal whose heart has stopped from a previous asphyxia, the œdema is even worse.

Experiment of February 20, 1905.—Adult male cat. Ether. Tracheotomy.

2:28 P. M. Put cannula in central end of right carotid, connected with manometer, and began blood pressure tracing.

2:35. Disconnected cannula and drew off 60 to 70 c.c. of blood from the right carotid; then again connected cannula with manometer.

2:50. Tied aorta and put cannula in central end. Bandaged abdomen and elevated posterior end of cat. Kept up artificial respiration.

3:10. Tied inferior vena cava and put cannula in its central end, so as to be able to draw off blood from right heart and restore it to pressure bottle if necessary.

3:12. Tied right subclavian artery and vein.

3:15. Tied left subclavian artery and vein. Corneal reflex still present.

In the interval, the vago-sympathetic trunk was stimulated in the neck, and both central and peripheral effects obtained.

3:33. Began injecting mixture of one part defibrinated cat's blood and ten parts of warmed Locke's solution. Allowed about 10 c.c. to run in.

3:35. Corneal reflex gone. Swallowing movements present. Pupils very much constricted (showing activity of third nerve center). Much liquid (largely saliva?) flowing from nose.

3:48. Swallowed. Heart beating strongly.

Central and peripheral effects of stimulation of vagus, and dilation of pupil on stimulation of sympathetic in neck, obtained several times.

4:06. Began injecting blood mixture.

4:13. Stopped injection. Eyelid reflex back in left eye, but not in right eye. Pupil of left eye less dilated than right (whose carotid artery is tied).

4:15. Lid reflex got in left eye distinctly by touching upper or lower lid. Corneal reflex absent. Secretion still rapidly escaping from nose and mouth.

4:18. Lid reflex increased in left eye, but left pupil is now dilated as widely as right. Pupils soon became insensible to light, although the corneal reflex persists for some minutes.

4:27. Began injecting blood mixture again. Corneal reflex still present, and

persistence of vagus effects, both peripheral and central, although latter gave rise of pressure instead of fall.

4:42. Stopped injection. Artificial respiration stopped to see if any asphyxial rise of blood pressure would occur. No rise of pressure, or very little. No lid reflex in eye.

4:44. Started artificial respiration again. Slight rise of blood pressure.

4:45. Began injecting again. Great rise in blood pressure and increase in force of heart beat.

4:49. Stimulated upper end of right and left vago-sympathetic nerves. The already widely dilated pupils dilated still more.

It was seen that, soon after the recommencement of artificial respiration, intense pulmonary œdema came on, and frothy, bloody liquid rose in the tracheal cannula. The tissues of the neck also seemed rather suddenly to become œdematous. Apparently, the short period of asphyxia had so injured the cells of the capillaries that they became more permeable to the liquids of the blood mixture. There was no trace of œdema in the neck before, and the circulation had never been totally interrupted before the asphyxia.

The Conditions to be Fulfilled in an Intra-arterial Injection.—From a consideration of the conditions necessary for the restoration of the excised heart, we may gain some idea of the conditions which must be fulfilled in order to bring about the restoration of the heart *in situ*. To be efficient, the injected fluid must pass through the coronary arteries, and maintain therein a pressure sufficient to cause contractions of the heart. It is not necessary for the injected fluid to enter the chambers of the heart although it has been shown that the pressure of blood in the chamber will cause some activity of the mammalian heart. The practical problem is to inject the fluid into the aorta in such a way that it shall go through the coronary arteries.

We have accomplished this result by means of a sound near the lower end of which is a distensible rubber sac. The sound is introduced through one of the carotids into the aorta so that the end is between the opening of the innominate and the semilunar valves. The rubber sac is distended by means of a syringe connected with it so as to occlude the aorta above the end of the sound. The injected fluid is then forced into this confined space against the semilunar valves, and through the coronary vessels. The fluid is gradually withdrawn from the distended sac, and the blood allowed to flow past it through the aorta as the heart begins to beat. The sound is finally withdrawn, the heart continuing to beat spontaneously.

The fluids best adapted for injection are defibrinated blood and serum diluted with one to five volumes of 0.9 per cent. sodium chloride solution, and the milk preparation described in a previous paper.[59] There is much less œdema when the blood and its dilutions are used than when the fluids containing the inorganic salts alone, or with the addition of dextrose, are employed for injection.

More recently Herlitzka[60] has accomplished resuscitation of the heart *in situ* in four rabbits by injecting fluid (Locke's solution to which adrenalin chloride was added) into the aorta through a sound introduced into it through the carotid artery. He used no arrangement for blocking the aorta. To reduce the pressure in the right auricle, blood was withdrawn through a urethral cannula introduced into it through the right jugular vein. Under these conditions, Herlitzka says that the path of least resistance lies through the coronary vessels rather than through the systemic circulation, to the right auricle. The heart, as a consequence of the establishment of the coronary circulation, begins to beat.

The necessity of maintaining a suitable blood pressure in order to maintain the activity of the heart *in situ,* even when it has not been previously stopped, or when it has been beating regularly and well for a considerable time after such stoppage has not been sufficiently recognized, and we quote the following experiments as bearing on this point.

The first experiment shows the effect of increasing the blood pressure, without any other remedial agency, on a heart which had rapidly weakened under low blood pressure, and also the beneficial effect of clamping the aorta, thereby increasing the blood pressure, in starting the heart by direct massage.

Experiment of March 20, 1905.—Cat. Ether. Artificial respiration.

3:15 P. M. Head arteries occluded.

3:22:30. Stopped artificial respiration. Natural gasps also stopped.

3:24:45. Again started artificial respiration. Pupils are dilated. Massaged heart (with unopened chest) and kept up artificial respiration. Heart did not start, nor was any sign of life restored, although the massage was vigorous and continued for a long time. Then opened chest and massaged heart directly. Heart did not start. Then clamped thoracic aorta and massaged heart directly.

3:37. Heart started under massage. If massage is intermitted heart stops

[59] *Amer. Jour. of Physiol.,* 1907, xviii, 8.

[60] Herlitzka, *Arch. ital. de biol.,* 1906, xliv, 93.

after a few beats. It is necessary to keep up massage until the heart is beating well (so as to fill the coronary arteries we may suppose). It then went on beating vigorously without an attention. Artificial respiration kept up all along.

4:07:30. First sign of life seen (observations made at very short intervals on eye reflexes, ear reflexes and so forth) namely, slight twitching of tongue and immediately after slight twitching of right corner of mouth. Then immediately a good strong respiratory gasp, involving the shoulders. More movements of the same kind succeed and increase in strength.

4:25. Corneal and lid reflexes back; also light reflex.

4:30. Stimulated left vago-sympathetic. Heart stopped, left eye bulged greatly, pupil dilated. No effect on right eye when left vago-sympathetic was stimulated.

Heart beating well. No doubt the experiment could have been continued, but we wished to see whether, when the aorta was gradually released, the heart would still continue beating.

4:31:30. Partially released clamp on aorta, and then entirely. Elevated hind end of cat.

4:33. Heart is slowing down and weakening. Corneal reflex gone. Pupils markedly dilated.

4:34. Compressed aorta again. Heart at once accelerates and improves in beat.

4:36. Released aorta again. Heart gets weaker. Elevation of hind end has little effect, except that heart fills better.

4:38. Clamped aorta. Heart had almost stopped. Ventricle soon gets stronger, but heart was too exhausted now and experiment was stopped.

The second experiment quoted shows the effect of gradually opening up the abdominal circulation by moving the clamps lower and lower until the splanchnic vessels are reached.

Experiment of March 29, 1905.—Half grown female cat. Ether. Tracheotomy. Artificial respiration. Thorax opened.

1:40:15 P. M. Clamped aorta just above intercostals.

1:41. Clamped head arteries.

2:01. Released head vessels, leaving clamp on aorta. Heart stopped for a beat or two when clamp was taken off of head arteries, but afterwards started up.

2:06. A gasp.

2:06:20. Another gasp. Then respirations go on regularly, five in the minute.

2:08. Respirations six a minute. Both fore limbs, ribs and neck move in the respiration and in fact all the muscles which have circulation. Pupils still dilated to maximum. No eye or ear reflex.

3:18. Strong light reflex. Contraction of pupil.

3:20. Stimulated left phrenic nerve. No contraction of diaphragm. Repeated; same result. Stimulated the diaphragm; fair contraction. Stimulated intercostal muscles; got contraction. Diaphragm does not contract in the natural respiration induced by stopping the artificial. Fore limbs quite excitable reflexly, and extended.

3:25. Stimulated upper end of brachial nerve. Strong reflex contraction

of the opposite fore limb, and of neck on both sides. Stimulation of peripheral end causes strong contraction of the limb.

3:31. Partially released the aorta. Heart soon begins to weaken and slow. Clamped aorta again in thorax; heart improved.

3:35. Clamped abdominal aorta just below the diaphragm above all branches given off below the diaphragm.

3:37. Took clamps off the thoracic aorta. Heart goes on beating as before.

3:38:30. Put clamp on abdominal aorta above the kidneys. Took off upper clamp. Liver is now getting plenty of blood, becoming red at once; also intestines. Heart goes on beating as well as before. Head respiratory movements are going on. Pupils well contracted but no light reflex.

3:43. Slight spasms, after which the pupils are dilated.

3:44. Snapping movements of jaws. Pupils are still widely dilated although not so widely as before. Clamped abdominal aorta above level of the renal vessels.

3:45. Took off upper clamp. The intestinal vessels are widely dilated and filled with blood. Spasms of forelimbs.

3:47. Heart is irregular since last removal of clamp. Now took off the lowest clamp and compressed hind limbs. Heart gets slower and weaker.

3:50. Now clamped thoracic aorta about level of middle intercostals. Heart immediately began to beat more rapidly but soon got slow again.

3:55. Stopped artificial respiration. No spasms. The intestine is not executing any movements. Stimulation of intestines has no effect. The same is true for urinary bladder, which is already strongly contracted and empty, for ureters, Fallopian tubes, and for muscle of abdominal wall.

The slow but certain failure of the heart when the blood pressure is lowered is shown in the third experiment quoted.

Experiment of March 30, 1905.—Cat. Ether. Artificial respiration.

10:48 A. M. Opened chest.

10:52. Clamped head arteries.

10:53. Clamped aorta below origin of left subclavian.

11:17:30. Released head vessels. Heart is beating poorly. Massaged it occasionally. Heart beats poorly, fibrillating a little and requiring occasional massage to keep it going until 11:57 A. M.

11:57. Heart now beating well. The animal continued in good condition, the reflexes returning and the heart continuing strong.

12:44. Corneal reflex back.

12:53. Took clamp off aorta. At once the nose and tongue which have been red become pale and the pupils dilate somewhat, especially the left.

12:55. Light reflex present. Gasping respirations start and now go on regularly, eighteen a minute. The previous apnœa, therefore, was associated with a good blood supply to the head apparently, as the blood pressure must be lower now with the whole animal under circulation after anæmia of the secondary vaso-motor centers in the dorsal and lumbar cord.

1:32. Circulation still going on. Stimulated sciatic. Fair contraction of corresponding hind limb.

1:40. Pupils found widely dilated. Heart stopped some time ago. Massage.

1:42. Heart beating feebly.
1:45. Abandoned experiment.

In none of these experiments could it be objected the heart had not recovered after starting by direct massage, or after the weakening which is such a constant accompaniment of cerebral anæmia. The chemical conditions were undoubtedly good, so far as nutritive value of the blood and its content of inorganic salts and oxygen were concerned. The physical condition of the heart was also good. But neither the blood nor the heart itself was able to overcome the wide effects of disturbing one physical factor in the problem—the blood pressure. This point will be considered again in the portion on the resuscitation of the vaso-motor mechanism.

Injection into Coronary Arteries.—The action of the various fluids on the excised mammalian heart, together with the conditions of its acitvity,[61] has been discussed in another paper. Blood is, of course, the best. Next to this come dilutions of blood with salt solution, the serum and dilutions of serum. Not very far behind serum comes milk prepared as follows: Milk was diluted three or four times with 0.9 per cent. sodium chloride solution after precipitation of the caseinogen with hydrochloric acid and making the filtrate slightly alkaline with sodium carbonate. Solutions of the inorganic salts are far inferior to any of the above. To give the best results, the fluid should, of course, be at the normal temperature of the blood.

The comparative effects of Locke's solution, and of a mixture of blood plus sodium chloride solution, on the same heart at varying times after its excision are shown in the accompanying protocol. It will be noted that, as the interval after death grows longer and the heart ceases to nourish itself well, the fluids become more nearly equal in their ability to elicit beats. It may be that the effects produced by the fluids at this time were purely mechanical, and such as might be produced by perfusion of the heart with mercury, oil or hydrogen gas.[62]

Experiment of February 27, 1905.—Heart of the cat used on February 20 was still in the ice box. On February 20 it had been isolated immediately after it

[61] Guthrie and Pike, *Amer. Jour. of Physiol.*, 1907, xviii, 14.

[62] Magnus, *Arch. für exper. Path. und Phar.*, 1902, xlvii, 200. Sollmann, *Amer. Jour. of Physiol.*, 1906, xv, 121. Guthrie and Pike, *ibid.*, 1907, xviii, 14.

stopped beating and perfused with Locke's solution made with calcium chloride not corrected for water of crystallization. It immediately began to beat and continued beating well. Then stopped perfusion and allowed the heart to cease beating. This it did in less than ten minutes. Then perfused with Locke's solution in which the amount of calcium chloride added had been corrected for water of crystallization. It began to beat at once and continued as well as with the other Locke's solution. No difference could be seen.

Then stopped perfusion and let the heart stop beating. Now perfused with a mixture of one part cat's blood to ten of sodium chloride solution. It beat more strongly than with either of the Locke's solutions. The pressure in all these perfusions was about sixty mm. Hg. in the perfusion bottle and temperature of liquid in the bottle and that of the heart was from 30 to 32° C. Put heart in the ice box. Sixteen hours later took it out and perfused again with Locke's solution under the same conditions of temperature and pressure. At end of about fifteen minutes it began to beat, the ventricles a little below the groove beating more strongly than the auricles. The beats were much feebler than on the previous day. Tried now perfusion with the blood mixture—same mixture as was used the day before. Got the same kind of beats as with Locke's solution. No noticeable difference in strength.

On February 23, forenoon, perfused the heart again with a mixture of cat's blood not perfectly fresh. No beats appeared even after two or three hours.

Put the heart again in the ice chest and kept it there until February 27 forenoon. It had now a slight putrid odor.

February 27, 1 P. M. Perfused it with Locke's solution. No beats whatever could be gotten. Perfusion kept up more than one-half hour.

5 P. M. Perfused heart with a mixture of cat's blood, fresh, and Locke's solution, one part of blood to eight of Locke's solution. No beats could be gotten, not even the feeblest. The same blood mixture causes good beating of fresh isolated heart of the cat obtained one hour after it had stopped beating *in situ.*

The Effect of Adding Drugs to the Injection Fluid.—Adrenalin and suprarenal extract have been injected in resuscitation experiments by a number of workers, probably first by us, and very soon after by Crile. Adrenalin is of slight value in cases where the thoracic aorta is occluded, unless massage is also used. With massage, when employed without occlusion of the thoracic aorta, adrenalin is of considerable value. Care must be taken not to use excessive quantities of the adrenalin, in order to avoid overstimulation of the heart and consequent fibrillary contractions.

We have tried the effect of direct intra-muscular injections of suprarenal extract upon the heart in one experiment.

Experiment of April 19, 1905.—Adult cat. Killed by etherization and bleeding at 4:47 P. M. Heart left *in situ.* Injection of Locke's solution into the aorta beginning at 5:04. The heart was kept beating until 5:22, when emboli were observed in coronary arteries near base of ventricle.

The suprarenal glands of a cat used in a previous experiment on this day were removed and ground in a mortar with Locke's solution.

5:35. Now injected suprarenal extract into the muscle of the ventricles. Strong contractions follow without diastole.

Such a result would manifestly be of no value in an attempt at resuscitation of an entire animal.

The effect of adrenalin when added to the circulating fluid when the heart is beating feebly is shown in the protocol of the experiment of February 27, 1905, cited above. An early experiment on adrenalin shows the uselessness of unconfined injections, even when adrenalin is added, and the impotency of adrenalin chloride itself in starting the heart.

Experiment of November 10, 1902.—Dog of 16 pounds. Given 8 c.c. of 0.2 per cent. morphine sulphate.

3:15 P. M. Anæsthetized with A.C.E. mixture. Tracheotomy.

3:25. Drew off 100 c.c. of blood. Connected manometers with carotid artery and external jugular vein.

3:55. Arterial pressure good. Pressure in right external jugular at base line or negative. Began opening chest.

4:01. Artificial respiration. Stimulated heart directly with induced current one minute. Marked fibrillary contractions result.

4:05. Respiratory movements still persist.

4:07. No pulse visible. Respiration ceased.

4:08. Heart still fibrillating. Applied adrenalin on surface.

4:14. Artificial respiration. Injected 10 c.c. of a mixture of 100 c.c. 0.9 per cent. sodium chloride and 1 c.c. of 1 to 1000 adrenalin chloride.

4:15. Heart still fibrillating.

4:16. 10 c.c. adrenalin injected.

4:17. 10 c.c. adrenalin injected.

4:18. Pressure unchanged in jugular vein. Injected 10 c.c. adrenalin.

4:19. 42 c.c. adrenalin injected. Ran in with very little pressure.

4:22. Pulse 130 a minute and feeble.

4:26. Stopped artificial respiration.

4:37. Injected 250 c.c. adrenalin solution.

4:39. Fibrillation of heart still feeble. No indication of rigor. Experiment discontinued.

Digitalein, which was used in rabbits only, gave results similar to those following the administration of adrenalin. The effect was more enduring than that of adrenalin, and the drug seemed, therefore, to be better adapted to the purpose.

Barium chloride was employed in a few experiments, but was abandoned as too dangerous and too uncertain. The regulation of

the dose is difficult and the secondary injurious action upon the walls of the blood vessels is a serious objection to its use.

Hydrogen peroxide, mixed with adrenalin and Locke's solution, has been used in successful resuscitations, but it is impossible to say in what degree the success was due to the peroxide.

Bandaging the limbs and abdomen, and particularly the latter, is of considerable value, owing to the intitial increase in the return of the blood to the heart in cases of hemorrhage, but it is of doubtful value where there has been no hemorrhage. When the blood volume is undiminished, it is relatively easy to produce over-distension and standstill of the right auricle by increasing the return of blood to it. Of course the diminution in the total vascular capacity tends to raise the blood pressure. The maximum increase in pressure produced by any ordinary bandaging is, of course, distinctly less than that produced by compression of the aorta.

The position of the body is of much importance, particularly where massage alone without intra-vascular injection is employed. We have thought that the advantage which experiment has clearly shown to be connected with the left lateral position might be associated with the easier return of the blood to the heart and possibly with a more favorable position of the ventricles for massage. The optimum position, as a rule, is attained by placing the animal on the left side and slightly elevating the posterior part of the body. When there has been no hemorrhage too great elevation of the posterior part of the body may easily cause over-distension of the right auricle and make resuscitation more difficult. In a number of experiments an increase of venous pressure corresponding to from 25 to 75 mm. of blood was sufficient to stop the right auricle. For example, in the experiment of March 1, 1905, quoted above, closing the outflow tube in the inferior vena cava stopped the auricular beats without affecting the rhythm of the superior vena cava.

Drugs introduced into the circulation can act upon the heart only when carried to it in the blood stream. It is obviously useless to try to start the heart which has completely stopped by subcutaneous injections of drugs. The addition of drugs which have a local action on the heart to fluids injected into the aorta may well have a beneficial effect. In case of the quiescent heart, drugs which

may beneficially affect the heart directly through its central nervous mechanism, *e. g.*, accelerator mechanism, or indirectly by raising peripheral vascular resistance can only exert their action if cardiac massage is combined with injection of fluid containing the drugs. Adrenalin chloride or any other drug which will constrict the arterioles, will be of service in increasing the arterial pressure and filling the coronary vessels.

In general, the mechanical methods for raising blood pressure are surer and more easily controlled than the methods involving the use of drugs. This agrees with Crile's observations on the treatment of surgical shock.

Electrical Stimulation of the Cardiac Nerves and of the Heart.—It has been shown by one of us (S.)[63] that stimulation of the augmentor nerves in the frog is capable of rousing a completely quiescent frog's heart from standstill.

Hering[64] has recently stated that rhythmical beats of the quiescent mammalian heart can be caused by stimulation of the nervi accelerantes. We have often seen beats produced in the hearts of cats and dogs by mechanical and electrical stimulation of the stellate ganglion or accelerator fibers. But we have not been able to cause complete resuscitation or restoration and maintenance of blood-pressure, which is a necessary condition for complete resuscitation by stimulation of the accelerantes alone.

We submit two condensed protocols of experiments showing the effect of mechanical stimulation of the accelerators. In the first one quoted, there can be little doubt that the heart had entirely ceased beating before the autopsy was made. Moreover, if such beats had persisted, they would have been noticed while the chest was open and the heart exposed. We regard this experiment as a demonstration that the heart may be caused to beat by stimulation of the accelerators after it has become completely quiescent.

Experiment of November 18, 1905.—Cat. Occlusion of cerebral arteries for twelve minutes. Cerebral circulation restored at 11:56 A. M. Spinal cord cut during anæmia. Artificial respiration maintained until 1:10 P. M. No spontaneous gasps occurred as long as cat was watched. On return to laboratory after lunch, there was every indication of death. No heart beat perceptible.

[63] Stewart, *Jour. of Physiol.*, 1893, xiii, 125. For other references see Stewart, *Amer. Jour. of Physiol.*, 1907, xx, 407.

[64] Hering, *Arch. f. d. gesam. Physiol.*, 1906, cxv, 354.

No respiration. Autopsy at 2:25 P. M.; while stripping fascia and pleura from cephalic vessels, the right auricle beat vigorously once. It beat again when the edge of it was pinched, with the forceps. Five beats were obtained by pulling at the fascia at some distance from the heart. Further attempts were not made. Ventricles did not contract.

While such a long time after heart stoppage did not elapse in the second experiment, we give the protocol, since it shows more specifically than the first the effects of mechanical stimulation of the accelerators, and the effects in this case extend to the ventricles as well as the auricles.

Experiment of April 19, 1906.—Large adult cat. Killed by etherization and bleeding at 3:45 P. M. Heart *in situ*. Artificial respiration employed. Pulmonary circulation left open.

4:00. Mixture of one-third defibrinated blood and two-thirds 0.9 per cent. sodium chloride solution injected into aorta. Auricles began to beat shortly after. The heart beat for a time, then ceased entirely or went into fibrillary contractions. Luciani's groups appeared before final cessation.

4:19. Heart completely quiet. Pinching stellate ganglion of either right or left side causes a beat of the left auricle and ventricle. Right ventricle not observed. A separate beat follows every pinch.

The possibility of maintaining heart beat and blood pressure by electrical stimulation must therefore be admitted, and the work of Floresco,[65] while it must be received with some caution at the present time, may lead to important applications in the near future. The work of Mathews and Jackson[66] on the excitation of the heart in standstill produced by magnesium salts and of Floresco in standstill produced by asphyxia, showed the possibility of using this method in practical resuscitation and is worthy of investigation, but we have not made any experiments on it.

Certain variations from the classical effects of stimulation of the cardiac nerves have appeared in the course of the experiments. For example, in the experiment of February 14, 1905, stimulation of the accelerators caused no contraction of the heart in a small young dog whose blood had largely been replaced by Locke's solution, although the interval elapsing between the time of stoppage of the heart and the time of stimulation was not as long as the intervals after which successful results were obtained in other experiments. Later on in the same experiment, when the heart was

[65] Floresco, *loc. cit.*

[66] Mathews and Jackson, *Amer. Jour. of Physiol.*, 1907, xix, 5.

being perfused with defibrinated blood, the stimulation of the vagus caused marked strengthening of the heart contractions.

Experiment of February 16, 1905.—In a similar experiment on February 16, 1905, in which about 500 c.c. of blood had been removed from the carotid artery, stimulation of the central end of the cut right vago-sympathetic nerve, the left being intact, produced acceleration of the heart. Stimulation of the peripheral end of the same nerve produced still more marked acceleration. The latent period before acceleration began was remarkably long, and the acceleration increased as the stimulation was continued. The beats seemed to become stronger also. On injecting a mixture of two parts Locke's solution and one part of blood, the heart became stronger and the blood pressure rose. Stimulation of the peripheral end of the right vago-sympathetic nerve caused practically no effect; a subsequent stimulation caused a primary acceleration followed by some inhibition, and a slight fall in pressure.

The other (left) vagus trunk was then divided. Stimulation of its peripheral end caused inhibition of the heart and a fall of pressure. The first stimulation of the central end caused a rise of pressure. Subsequent stimulation of the central ends of both right and left vagi caused a fall in pressure. During the remainder of the experiment (about an hour) stimulation of the central ends of the vagi nearly always caused a rise in pressure. In four separate counts the rate of the heart (50 in 21½ seconds) was exactly the same before and during stimulation, although a good rise in blood pressure occurred after a somewhat long latent period in each instance.

In the experiment of March 1, 1905, in which artificial circulation was kept up, stimulation of the peripheral portion of the right vago-sympathetic caused no inhibition of the beats of the auricle and superior vena cava, but rather an acceleration.

A rise of blood pressure has frequently been noticed on stimulation of the central end of the dog's vago-sympathetic, but under normal conditions stimulation of the central end of the vagus in cats has given, without a single exception, a fall in blood pressure, with inhibition of the heart if the other vagus was intact.

The Fibrillary Contractions and the Means of Overcoming Them.—A troublesome feature of resuscitation of the heart is the fibrillary contractions which often appear as a sequel of massage or intra-arterial injections. Magrath and Kennedy[67] state that they have repeatedly seen the cat's heart recover from fibrillation after being in that condition for many minutes. D'Halluin[68] successfully added potassium chloride to the injected blood or other fluid to overcome the fibrillation of the heart. Herlitzka[69] in some

[67] Magrath and Kennedy, *loc. cit.*
[68] D'Halluin, *loc. cit.*
[69] Herlitzka, *loc. cit.*

cases used adrenalin chloride for this purpose, with considerable success. In experiments on the excised heart, where injection was made directly into the coronary artery, we had little difficulty with fibrillation. We have often encountered fibrillary contractions during massage of the heart *in situ,* but they have ceased if massage was continued and a sufficient blood pressure attained. For example, in the experiment of March 23, 1905, the protocol of which has been given above, fibrillation of the ventricles occurred after starting the heart by direct massage, but disappeared as the massage was continued from time to time, and the blood pressure increased. Sometimes it has been impossible to get the heart to beat regularly after the fibrillations have once begun. These fibrillary contractions and other irregularities of rhythm are, as a rule, more likely to occur when the salt solutions, *e. g.,* Locke's, are employed for injection or perfusion than when a proteid containing fluid, such as defibrinated blood, dilute serum, or the milk preparation above described is used. The most successful means of overcoming these contractions when they have appeared is, in our experience, an adequate circulation of the blood through the coronary vessels.

The following experiment shows that recovery from fibrillary contractions may occur suddenly.

Experiment of May 16, 1905.—Adult male cat. Ether. Tube in larynx in which there was an occlusion of twenty-one minutes; the heart stopped about sixteen minutes after the release of the head arteries. Massage of the chest was tried, to no purpose. About fifteen minutes after the heart stopped, the thorax was opened, the aorta clamped and the heart massaged directly. Heart entirely motionless at the time the chest was opened. In five minutes the auricles were beating well, and the ventricles fibrillating. The auricles began to beat very soon after massage was begun, but the ventricles not for some time. Suddenly, seven minutes after the chest was opened, the ventricles began to beat well. No further massage was needed throughout the experiment, which was continued for five hours longer; when it was discontinued, the heart was still beating excellently.

Resuscitation of the Regulative Mechanisms of the Heart.—The question is an interesting one whether the cardiac regulative mechanisms are rsuscitated at the same time as the automatic beat. The answer is, that in general the central innervation of the heart, both accelerator and inhibitory, is in abeyance for a longer or shorter period after the spontaneous beat of the heart has been restored,

the length of this period depending on the time of occlusion. The same is true of the local regulative mechanism which we have treated of in a previous paper.[70] For example, while in the normal heart the rate is diminished by increase of coronary pressure and increased by diminution of pressure in the heart, during resuscitation there is a period when increase of pressure is accompanied by increase in the rate and vice versa, just as happens in the excised heart. This fact suggests that the local mechanism which in the normal heart even in the absence of extrinsic innervation causes the response of diminished rate to increased pressure and increased rate to diminished pressure has not yet been restored. After long periods of asphyxia no restoration may ever take place. As regards the mechanism, whatever it may be, which normally coördinates the contraction of the two ventricles and renders it synchronous, it seems to be resuscitated as soon at any rate as the power of the ventricles to beat, since when the ventricular beat appears it is found to start synchronously on the two sides. This is in favor of the view that no specific mechanism is concerned in this coördination.

Arhythmia of auricles and ventricles and Luciani's groups are fairly common phenomena in the resuscitation of the heart *in situ* or in perfusion of the excised heart. Arhythmia of the auricle and ventricles was noticed in the experiment of March 1, 1905, already quoted. In the same experiment, a more rare phenomenon was observed, namely, arhythmia of the superior vena cava and the auricles.

In many of the experiments the heart, during the resuscitation following cerebral anæmia, is generally beating as fast as hearts whose vagi have been cut, although stimulation of the peripheral end of the vagus stops or slows them. This, we believe, indicates that the vagus center has not recovered its tone, although its endings are intact. The significance of this will be considered further in the section on the cardio-inhibitory center. During the inactivity of the vagus center, however, asphyxial slowing of the heart occurs as usual. Since we have shown that asphyxial slowing of the heart may result after section of both vagi and division of the cervical spinal cord, we conclude that the action may be local, and not necessarily central.

[70] Guthrie and Pike, *loc. cit.*

We[71] have called attention in another paper to the peculiar double beat appearing in the tracing of the excised heart when the pressure of injection is low. A similar double beat frequently occurs at a certain stage in the occlusion period, as is shown in the following experiment.

Experiment of May 29, 1905.—Adult male cat. Ether. Tube in larynx. Pulse (under ether before experiment) 207 a minute. Respiration about 60 a minute.

2:50 P. M. Occluded head arteries in usual way.
2:50:15. Respiration rapid and shallow.
2:50:25. Corneal reflex gone.
2:50:40. Natural respiration stopped. Started artificial respiration.
2:51:45. Pupils same as before occlusion, not at all dilated.
2:52:30. Pupils dilating.
2:53. A gasp (secondary series).
2:53:20. Pulse had double beat usually seen at this stage.
2:53:30. Pulse 216 in the minute.
2:54:05. Double beat of pulse gone.
2:54:30. Gasps still going on.
2:57:30. Gasps cease. Pupils at maximum dilation.

From a study of the above excerpt from the protocol of the experiment, it will be seen that the double beat appears only during the time in which the cardiac and other bulbar centers remain active, and that it ceases at about the time that the inhibitory center fails. Since the heart goes on with machine-like regularity after the total failure of the bulbar centers, there seems little doubt that the double beat is caused, in the case of the heart *in situ* by the extrinsic cardiac nervous mechanism, and since, furthermore, it appears during the time of the activity of the inhibitory mechanism, it is possible that this double beat is a phenomenon of inhibition.

Resuscitation of the Heart after Death from Other Causes.—From a considerable number of experiments we conclude that chloroform has the most deleterious action, the A. C. E. mixture coming next in order, and then ether. All our evidence tends to corroborate the current statement that chloroform acts very injuriously on the heart tissue. Morphine, when used subcutaneously in addition to any of the other anæsthetics, tended, as a rule, to make resuscitation less successful.

Hemorrhage complicated the result here as well as in asphyxia.

[71] *Amer. Jour. of Physiol.*, 1907, xviii, 24.

The hemorrhage was always carried out under general anæsthesia.

One cause of the greater difficulty of resuscitation attending death from hemorrhage is the absence of a sufficient volume of nutritive fluid. So we must first supply a certain volume of fluid of a suitable nature, and afterwards get it in motion. A fluid as good as serum or blood can only be obtained by transfusion. As we have not empolyed this method, adhering to blood dilutions and artificial liquids, it can not be stated definitely whether hemorrhage in itself, by sooner exhausting the supply of nutriment of the tissues than would be the case where the circulation is simply stopped, or by robbing the lymph unduly of water, renders resuscitation more difficult, even under the influence of a copious supply of transfused blood. Judging from our work upon the excised mammalian heart, the milk preparation which we then employed would be more suitable for intra-vascular injection after hemorrhage than the salt solutions.

Resuscitation after Electrocution.—Our first experiments on resuscitation after electrocution were made in 1901. They were not satisfactory because of the low voltage (110 volts, direct) of the currents available for the purpose. Sponges were connected with the ends of the wires and used as electrodes. One was placed on each side of the thorax of an anæsthetized dog. The circulation was but little affected even after some minutes.

Direct stimulation of the heart with induced currents caused delirium cordis. The restoration of the circulation afterward was not very successful, owing to the difficulty of overcoming the delirium. Cold, asphyxia and the methods generally recommended for the purpose were quite inefficient in our hands. Resuscitation appeared more difficult than after asphyxia, anæsthetics or hemorrhage. Later on, when Dr. Crile began a series of experiments on the same subject with a much more powerful instalment, we allowed the matter to drop.

SUMMARY.

Our results may be briefly summarized:

1. Blood, when defibrinated, soon loses its power to maintain the activity of the higher nervous centers, and its nutritive properties for all tissues quickly diminish.

2. Artificial fluids, as a substitute for blood, are not satisfactory.

3. The proper oxygenation of the blood is an indispensable adjunct in the resuscitation of an animal.

4. The heart usually continues to beat for some minutes after it ceases to affect a mercury manometer, and resuscitation of it within this period by extra-thoracic massage and artificial respiration is sometimes successful.

5. Resuscitation of the heart by direct massage is the most certain method at our command.

6. A proper blood-pressure is an indispensable condition for the continued normal activity of the heart.

7. Anæsthetics, hemorrhage and induced currents applied to the heart render resuscitation more difficult than asphyxia alone.

THE EFFECT OF INJECTED LEUCOCYTES UPON THE DEVELOPMENT OF A TUBERCULOUS LESION.*

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The purpose of the experiments which will be described has been to determine the effect of injected leucocytes upon the development of a tuberculous lesion. For the experiments the dog, which is somewhat insusceptible to tuberculosis has been selected for two reasons. Preceding studies have demonstrated methods by which it is possible to obtain sterile leucocytes in great quantity almost wholly free from the inflammatory irritant which has been used to cause their accumulation. Of equal importance for the purpose of the experiments is the fact that the development of the tuberculous lesions produced by injection of tubercle bacilli into the pleural cavity of the dog can be followed with considerable accuracy by percussion of the animal's chest.

The insusceptibility of the dog to tuberculosis has been exaggerated and its apparent immunity to the disease is doubtless dependent in part upon the fact that its habits do not expose it to infection. Freedom from spontaneous infection is not an accurate index of susceptibility, for the guinea-pig, in which tuberculosis develops with great readiness, is rarely subjected to spontaneous infection. Spontaneous tuberculosis in dogs has been studied especially by Jensen (twenty-eight cases), Cadiot (forty cases) and Eber[1] (eleven cases). The lungs are the primary seat of infection in a large proportion of the cases. The pleura and mediastinal lymphatic glands are implicated. Jensen has described the sarcoma-like appearance of tuberculous tissue in the dog and thinks that tuberculosis of various organs in this animal closely resembles the same lesion in cattle.

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[1] Eber, Lubarsch and Ostertags *Ergebnisse der allg. Path.*, 1897, iv, 859.

Injection of a suspension of tubercle bacilli into the pleural cavity of dogs causes tuberculosis which is almost constantly fatal. Fluid accumulates in the cavity; flat nodules of grayish white tuberculosis tissue are formed upon the pleura of the chest wall and diaphragm and occasionally upon the surface of the lungs. The lesion is bilat-

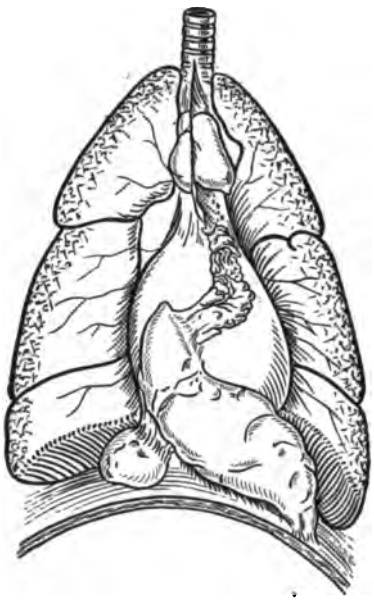


FIG. 1.

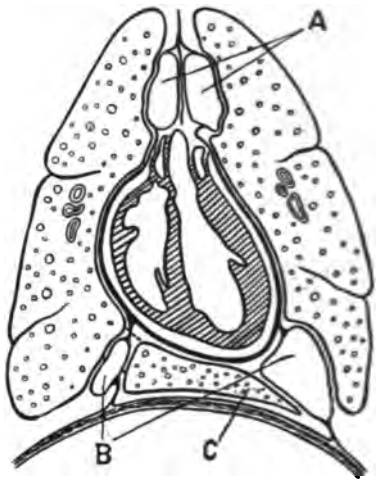


FIG. 2.

FIG. 1. Diagram to accompany Experiment 10, showing distribution of tuberculous tissue after inoculation of the right pleural cavity with tubercle bacilli. Tuberculous tissue occupies the mediastinum and subpericardial membranes; the substernal lymphatic glands are enlarged and tuberculous.

FIG. 2. Lesion represented by the preceding figure in section showing the membranes which extend from the pericardium to diaphragm and partially enclose a cavity into which fits a lobe of the right lung (*C*). *B*, tuberculous tissue in subpericardial membranes; *A*, enlarged substernal lymphatic glands.

eral and fluid accumulates in both the right and left pleural cavities, but the disease usually progresses more rapidly in the cavity which has received the injection. Rounded masses of firm gray-white tissue appear in the mediastinum (Fig. 1) and when the lesion is advanced may grow together, forming a continuous mass extending the whole length of the sternum. The tissue is at first succulent

grayish white and homogeneous, having an appearance suggesting sarcoma, but later becomes opaque and yellow where caseation occurs. Similar masses of tuberculosis tissue (Fig. 2), often continuous with those in the mediastinum, occupy the membranes which in the dog extend from each side of the pericardium to the diaphragm and enclose a cavity; into this cavity fits a lobe of the right lung. The mediastinal lymphatic glands (Figs. 1 and 2), situated behind the upper end of the sternum, quickly enlarge, become hard and undergo caseation.

Dissemination of tubercle bacilli occurs at an early period of the disease and ten days after inoculation tubercles may be found in the liver. After many weeks tubercles may be found in the lungs, spleen and kidneys. Extension by way of the lymphatic system occurs rapidly and evidence of tuberculosis appears successively in the retropleural glands just above the diaphragm, in glands near the duodenal part of the pancreas and in the retroperitoneal glands which are near the adrenals.

Death frequently occurs as the result of broncho-pneumonia due to secondary infection. After several weeks the animal may cough and there is abundant purulent discharge from the nose. Associated with this condition conjunctivitis and ulceration of the cornea may occur.

The experiments which have been described have been possible only because the course of the disease can be followed during life by percussion of the chest. In the normal animal standing in its usual position there is relative dullness two or three centimeters to the right of the median line caused by projection of the heart to the right. The median line of the animal is marked and the upper limits of relative and absolute dullness accurately measured immediately behind the fore-leg; figures thus obtained are accurate within less than half a centimeter and afford the only available means of measuring during life changes in the fluid and solid contents of the chest.

After injection of tubercle bacilli increase of relative dullness over the ventral part of the right chest is evident within one or two days and this impaired dullness increases continuously. Usually within a week or ten days absolute dullness makes its appearance.

Changes in the extent of dullness on percussion are referable in part to accumulation of fluid, in part to solid tuberculosis masses in the mediastinum and adjacent membranes. Observations made by puncture of the animal's chest and by autopsy indicate that absolute dullness is caused by the presence of fluid. Not infrequently in inoculated animals absolute dullness disappears although increased relative dullness persists and the disease continues with undiminished severity. In such instances disappearance of absolute dullness is doubtless due to absorption of pleural effusion.

The following experiments illustrate the effect on dogs of intrapleural injection of the strain of tubercle bacillus employed in the greater number of the experiments which will be described. The organism had the characters of the human type of tubercle bacillus and was of only moderate virulence, killing guinea-pigs by intraperitoneal injection after from three to four weeks; it did not kill rabbits after injection into the peritoneal cavity, but caused their death six or seven weeks after intrapleural inoculation.

EXPERIMENT 1.—Dog, wt. 4,150 grm. Into the right pleural cavity was injected 0.5 c.c. of a suspension of B. tuberculosis. Relative and absolute dullness over the right side of the chest gradually increased so that at the end of about a month relative dullness measured 8.5 cm. and absolute dullness 6.6 cm. Below the skin at the point of injection a nodular mass fixed to the chest wall made its appearance two weeks after inoculation, increased in size, and opened spontaneously; it remained as an open wound until death which occurred with increasing emaciation at the end of 50 days after inoculation.

Autopsy.—Each pleural cavity contains about 150 c.c. of almost clear yellow fluid which on standing forms a transparent coagulum. Upon both visceral and parietal pleuræ are flat, yellowish-white nodules. Masses of hard, partly caseous tuberculous tissue occur in the mediastinum and in the membranes extending from pericardium to diaphragm. The substernal lymphatic glands are greatly enlarged and caseous. The liver contains an immense number of miliary tubercles which occupy about one half the area of the section prepared for microscopic examination. The spleen and kidney contain tubercles in small number.

EXPERIMENT 2.—Dog, wt. 4,850 grm. Into the right pleural cavity was injected 1 c.c. of a suspension of B. tuberculosis. Dullness over the ventral part of the thorax on the right side increased so that at the end of two weeks the upper level of relative dullness was 7.6 cm. from the mid line; of absolute dullness, 3 cm. During this time the body weight had been maintained. The animal became thin and death occurred at the end of 20 days after the inoculation.

Autopsy.—The right pleural cavity contains 75 c.c. of almost clear yellow fluid; the left cavity contains 80 c.c. Upon the surface of both lungs are flat gray-white projections usually less than 1 mm. across. The mediastinum which is thickened contains large masses of newly formed hard grayish white tissue;

a similar mass is in contact with the diaphragm. The lymphatic glands below the cephalic end of the sternum are greatly enlarged and caseous. The liver contains an immense number of tubercles which occupy at least a third of the section for microscopic examination. The spleen contains tubercles; none are found in the kidneys.

Inoculation with 0.5 c.c. of a suspension of tubercle bacilli caused death in fifty days whereas twice this amount of the same suspension was more quickly fatal.

The leucocytes used for injection have been obtained from dogs by repeated injection of turpentine into the pleural cavity. One or two cubic centimeters of turpentine have been injected into the right pleural cavity; when after three days the resulting inflammatory exudate has reached a maximum a second similar injection is made. Fluid continues to accumulate and may be serous, seropurulent or purulent. Aspiration of this fluid is followed by accumulation of purulent exudate of which a third of the volume may be leucocytes.

Leucocytes obtained one or two days either after injection of turpentine or after aspiration are separated by centrifugalization from the serum of the exudate and twice washed by centrifugalization with normal salt solution. The leucocytes after removal of the overlying salt solution readily pass through the coarse needle of a syringe. The quantities injected represent volumes of leucocytes packed together by centrifugalization.

A coarse needle with blunt beveled point and with an opening at the side a short distance from the end has proved convenient for intrapleural injection. The skin is punctured with a sharp instrument and the needle of the syringe is inserted obliquely in such position that the beveled surface of the end is parallel with the chest wall.

Washed leucocytes obtained by the method which has been described cause a readily recognizable reaction when introduced into the right pleural cavity of a normal dog. Ten cubic centimeters of these cells cause an accumulation of fluid which is indicated by a broad area of relative and usually of absolute dullness over the dependent part of the cavity. This increased dullness reaches a maximum on the day following injection and rapidly subsides, disappearing after three or four days. The quantity of fluid which accumulates (indicated by the amount of dullness) and the dura-

tion of the reaction increases with the quantity of injected leucocytes.

The pleural cavity is not permanently altered by the reaction which occurs. In an animal which had received four injections of leucocytes (10 to 25 c.c.) at intervals of about one week the pleural cavities were found to be normal and the mediastinum and adjacent membranes delicate.

Series A.—The effect of injections of leucocytes upon thoracic dullness increased by inoculation with Bacillus tuberculosis.

One half cubic centimeter of a suspension of *Bacillus tuberculosis* was injected into the right pleural cavity of six dogs. Dullness on percussion over the right side of the thorax underwent an increase, exhibiting in different animals considerable variation in rapidity. Two animals (weighing respectively 5,050 and 7,250 grm.) in which the disease was allowed to pursue its course served as control. At the end of seven or eight days, when relative dullness was much increased and absolute dullness had made its appearance in all of the inoculated animals, leucocytes in quantities from twelve to twenty-five cubic centimeters were injected into the right pleural cavities of the remaining four dogs; the injections were repeated at intervals of about one week.

For extraneous reasons it was found necessary to discontinue injection of leucocytes at the end of one month after inoculation; at this time the area of dullness had in the four injected animals diminished considerably and was not much greater than that present before the onset of tuberculosis. It was thought possible that recovery might follow but examination of the chest on the fortieth day of the disease showed that in two animals there was increase of relative and reappearance of absolute dullness.

EXPERIMENT 3.—Dog, wt. 5,450 grm. The animal received three intrapleural injections of leucocytes; changes in thoracic dullness are indicated below. At the end of a week a small nodule was found below the skin at the point at which the inoculating needle had been inserted; ten days later leucocytes were injected into the nodule. It became smaller but finally broke upon the surface and remained as a discharging ulcer until death. Death occurred 57 days after inoculation.

Day of Disease.	Absolute Dullness.	Relative Dullness.	
1	—	2.7	Inoculated with tubercle bacilli.
9	3.0	3.9	14 c.c. leucocytes injected.
10	4.7	6.6	

12	—	4.3	
19	—	3.4	12 c.c leucocytes injected.
20	—	3.4	
27	—	5.3	10 c.c. leucocytes injected.
28	—	4.5	
29	—	3.8	
40	4.6	8.1	
58			Died.

Autopsy.—The pleural cavities are each distended with several hundred cubic centimeters of turbid fluid which compresses the lungs. The mediastinum and subpericardial membranes which are thickened and opaque contain large confluent gray white masses with a maximum thickness of 0.5 cm. The mediastinum is pouched to the right and much crinkled. Parietal and pulmonary pleuræ are thickened and opaque. The substernal lymphatic glands are moderately enlarged and partially caseous. The liver is large and contains an immense number of tubercles. An occasional tubercle is found in the lungs.

Experiment 4.—Dog, wt. 6,850 grm. The animal was inoculated into the right pleural cavity with 0.5 c.c. of a suspension of *B. tuberculosis.* A nodule formed in the skin at the point of injection. The progress of the disease during which leucocytes were injected three times is shown by the following table. Death occurred after 68 days.

Day of Disease.	Absolute Dullness.	Relative Dullness.	
1	—	4.0	Inoculated with *B. tuberculosis.*
9	4.0	5.6	25 c.c. leucocytes injected.
10	3.0	5.6	
12	—	3.7	
19	—	3.7	11 c.c. leucocytes injected.
20	3.3	7.0	
22	—	4.8	
27	—	4.8	10 c.c. leucocytes injected.
28	—	4.6	
40	—	4.6	
69			Died.

Autopsy.—The pleural cavities contain a large quantity of fluid which compresses the lungs. The mediastinum contains masses of newly formed tissue which is dense and fibrous and contains caseous patches. Masses of similar tissue occur in the membranes which extend from pericardium to diaphragm. The lungs are atalectatic and contain upon their surfaces and in their substance numerous tubercles. About one third of a section of liver consists of tuberculous tissue.

Experiment 5.—Dog, wt. 6,750 grm. The animal received into the right pleural cavity 0.5 c.c. of a suspension of tubercle bacilli. A nodule appeared at the point of inoculation about a week later. Leucocytes were injected into the nodule which subsequently diminished much in size. The animal received four injections of leucocytes into the pleural cavity, it became emaciated and died at the end of 89 days.

Day of Disease.	Absolute Dullness.	Relative Dullness.	
1	—	2.5	Inoculated with *B. tuberculosis.*
9	4.9	7.4	12 c.c. leucocytes injected.
10	2.0	3.9	
12	—	3.1	
19	—	4.1	22 c.c. leucocytes injected.
20	2.0	5.0	
22	—	4.2	
27	—	4.6	10 c.c. leucocytes injected.
28	—	4.9	
33	—	4.0	10 c.c. leucocytes injected.
40	2.8	6.8	
90			Died.

Autopsy.—Pleural cavities contain a large amount of fluid. The pleura is thickened and opaque and on its surface in places is a thin layer of fibrin. The

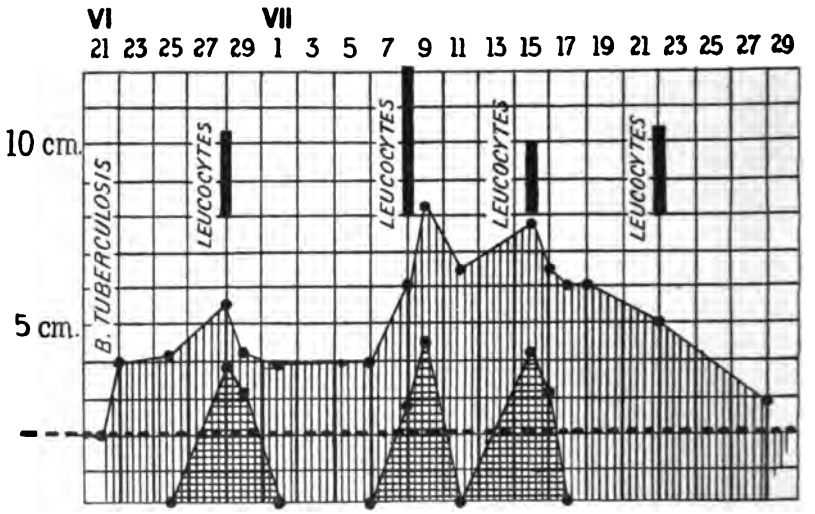


CHART I.—The progress of the disease in Experiment 6 and in subsequent experiments has been depicted by a chart which indicates the amount of absolute and relative thoracic dullness measured to the right of the mid line. Injection of leucocytes is indicated by heavy black perpendicular lines whose length (one square equals 5 c.c.) represents the quantity of cells injected. Relative dullness is represented by the lightly shaded zone; absolute dullness by the heavily shaded areas. In the normal animal there is no absolute dullness over the thorax to the right of the mid line but since projection of the heart to the right causes relative dullness two or three centimeters beyond the mid line a normal base line (dotted horizontal line) of relative dullness has been drawn for each animal. Weight is indicated by a dotted line at the upper part of each chart.

mediastinum is free from tuberculous masses save above the diaphragm where there is a mass of fibrous and caseous tissue; in the membranes which extend from pericardium to diaphragm are similar masses, that on the right being the larger. The mediastinal lymphatic glands are moderately enlarged and consist of caseous material surrounded by a fibrous capsule. The lungs and liver contain tubercles in immense number. In the liver they are surrounded by a thin fibrous capsule.

EXPERIMENT 6.—Dog, wt. 4,750 grm. Seven days after inoculation with *B. tuberculosis* as a time when relative dullness over the right chest had increased and absolute dullness had made its appearance leucocytes were injected. Three similar injections were subsequently given and at the end of 27 days dullness over the right chest was only slightly greater than that before inoculation; the changes are shown by Chart 1. During this period the body weight diminished slightly. The animal at the end of eight months is very active and apparently well, its weight being 2,050 grm. more than the weight at the time of inoculation. Percussion shows only a normal relative dullness over the right thorax.

Control Experiments.

EXPERIMENT 7.—Dog, wt. 5,050 grm. Control. The weight of the animal fell quickly after inoculation. Relative dullness over the right pleural cavity gradually increased and absolute dullness was present at the end of a week. Absolute dullness disappeared although relative dullness persisted until a short time before death. Death occurred after onset of cough and purulent nasal discharge at the end of 34 days.

Day of Disease.	Absolute Dullness.	Relative Dullness.	
1	—	3.1	Inoculated with *B. tuberculosis.*
8	4.5	6.0	
11	4.5	6.0	
15	—	4.5	
21	—	4.5	
25	—	6.1	
28	—	6.1	
35			Died.

Autopsy.—The animal is emaciated. In the right chest wall at the site of inoculation is a mass of partially caseous tuberculous tissue which projects upon the parietal pleura. Each pleural cavity contains only about 25 c.c. of fluid. The thickened and injected mediastinum and adjacent membranes contain tuberculous masses. The substernal, retropleural and retroperitoneal lymphatic glands are much enlarged and tuberculous. There is bronchitis and patches of broncho-pneumonia. The liver is enlarged, and exhibits fatty degeneration; small caseous tubercles are numerous.

EXPERIMENT 8.—Dog, wt. 7,250 grm. Control. After inoculation the body weight rapidly fell; relative dullness over the right pleural cavity increased slightly and at the end of a week absolute dullness had made its appearance but subsequently disappeared. Death occurred at the end of 35 days.

Day of Disease.	Absolute Dullness.	Relative Dullness.	
1	—	5.6	Inoculated with *B. tuberculosis.*
9	3.3	6.1	
12	3.3	6.1	
16	3.3	5.2	
20	3.3	5.2	
22	—	6.1	
29	—	6.1	
36			Died.

Autopsy.—The animal is emaciated. Each pleural cavity contains about 150 c.c. of turbid yellowish fluid. The mediastinum and subpericardial membranes which are intensely injected contain caseous masses. The substernal lymphatic glands and lymphatic glands near the duodenum are enlarged and caseous. The lungs contain deep red patches of broncho-pneumonia and there is bronchitis. The liver contains numerous small caseous tubercles.

The first injection of leucocytes into a pleural cavity containing effusion, indicated by absolute and increased relative dullness, has in two instances (Experiments 5 and 6) been followed within twenty-four hours by diminution of dullness, doubtless the result of absorption of fluid. In one instance (Experiment 4) fall of the level of dullness was delayed at least twenty-four hours whereas in one instance (Experiment 3) a well-marked increase of dullness preceded the disappearance of absolute dullness, relative dullness, probably due to the presence of tuberculous masses, persisting. Injections of leucocytes were repeated at intervals of a week or ten days, dullness not infrequently increasing between injections and falling after them. These changes are illustrated by Chart I, in which relative and absolute thoracic dullness are charted.

In this series of experiments, which were begun eight months ago, two control animals died at the end of five weeks; two animals receiving three injections of leucocytes lived about two months; a third injected animal, receiving four injections, lived three months, and a fourth animal receiving the same number of injections is living and well.

It is noteworthy that injection of a large quantity of leucocytes (20 c.c.) has been usually followed by marked increase of dullness with subsequent fall. The following experiments show the effect of leucocytic injections repeated more frequently and in larger quantity than those previously employed.

Series B.—The effect of leucocytes injected into the pleural cavity at short intervals and in large quantity during the course of tuberculous pleurisy.

Two animals were inoculated intrapleurally with 0.5 cubic centimeter of a suspension of *Bacillus tuberculosis.* During the first ten days changes of thoracic dullness pursued an approximately parallel course in the two animals, relative dullness gradually increasing and absolute dullness appearing. The injected animal died at the end of fifty-six days and the control animal was immediately killed for comparison.

EXPERIMENT 9.—Dog, wt. 5,800 grm. At the end of a week a nodular thickening had developed in the chest wall at the point of inoculation; leucocytes were injected into the nodule which after several weeks diminished in size. Leucocytes were injected into the pleural cavity ten days after inoculation and the animal received four injections within fifteen days. Mange made its appearance and was widely distributed upon the skin. The animal became thin and death occurred at the end of 56 days.

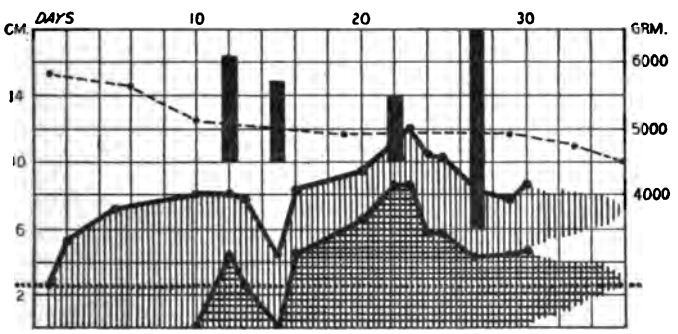


CHART 2. Experiment 9; injection of leucocytes.

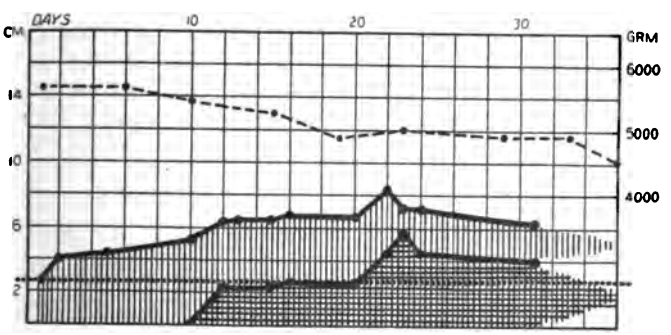


CHART 3. Experiment 10; control.

Autopsy.—The body is thin but not emaciated. The right pleural cavity is distended with several hundred cubic centimeters of almost opaque whitish fluid which compresses the lung. The parietal and pulmonary pleura is everywhere grayish white and thickened often to 1 mm. The left pleural cavity is also distended and the pleura is grayish white but less thickened than on the right side. The distribution of the lesions which are present is represented by Fig. 3; compare with Fig. 2 showing the lesions in the control. Upon the surface of the mediastinum which is thickened and leathery are several flat yellowish white elevations. At the junction of the subpericardial membranes and diaphragm on each side are small scar-like masses which on section are composed of grayish white tissue. The substernal lymphatic glands are moderately enlarged, measuring 1.4 cm. in long diameter. The lungs, which are much compressed, contain numerous tubercles. The liver contains a great number of large tubercles; tuberculous tissue occupies at least a third of a section for microscopic examination.

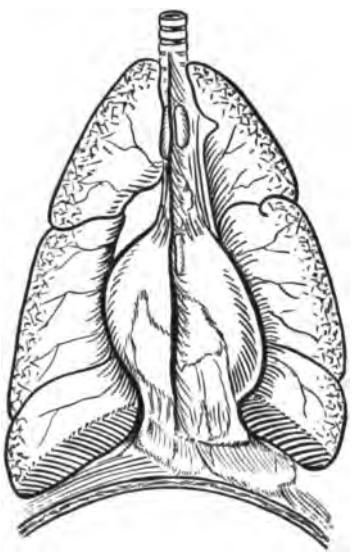


FIG. 3. Diagram of lesion of Experiment 9; compare with control represented by Fig. 1.

Control Experiment.

EXPERIMENT 10.—Dog, wt. 5,650 grm. Control. Ten days after inoculation a nodule made its appearance immediately below the skin at the site of injection; it gradually increased in size and broke two weeks later. There was cough beginning about ten days after inoculation. Weight diminished gradually. The animal was killed at the end of 56 days, for comparison with that of the preceding experiment.

Autopsy.—The right pleural cavity contains about 100 c.c. of turbid fluid; the left cavity contains about the same amount of fluid. The parietal and pulmonary pleuræ are not thickened. Situated in the mediastinum above the diaphragm and extending into the subpericardial membrane is a very large mass of grayish white succulent, in places, caseous tissue (see Figs. 1 and 2); a similar mass which is smaller occupies the subpericardial membrane on the right side. Figs. 1 and 2 show the situation of these tuberculous masses. A large mass of tuberculous tissue is situated in the posterior mediastinum above the diaphragm. The substernal lymphatic glands are greatly enlarged, hard and caseous, measuring 1.6 cm. in long diameter. The lungs contain no tubercles. The liver contains small scattered tubercles.

Whereas increase of thoracic dullness proceeded uninterruptedly in the control, the first intrapleural injection of leucocytes in the treated animal was followed by a fall of relative and disappearance of absolute dullness. A second injection three days after the first

was followed by an accumulation of fluid which showed no tendency to subside until a third injection was given. The fourth injection (30 c.c) was given with the hope of influencing favorably by a large quantity of leucocytes what appeared from the extent of thoracic dullness to be a very severe infection. Fluid showed little indication of decrease and death resulted about one month later. The generalized chronic pleurisy with effusion, which doubtless caused or hastened death, has not been observed in any of the untreated tuberculous animals and is probably referable to the injections of leucocytes which were repeated at unusually short intervals and in unusual amount; for repeated observations have demonstrated that the intensity of the inflammatory reaction which follows injection of leucocytes bears a relation to the quantity injected.

Although the injection of leucocytes did not prolong the life of the animal nor exert a favorable influence upon the course of the disease, comparison of the lesion with that of a control animal inoculated with the same suspension and killed after the same interval has shown that the local tuberculous process has been markedly retarded. In the control animal (Fig. 1) the mediastinum and subpericardial membranes are occupied by enormous succulent partially caseous masses and the lymphatic glands adjacent to the pleural cavities are greatly enlarged and caseous. In the treated animal (Fig. 3) there are small scar-like masses almost wholly composed of fibrous tissue in the same situations and the adjacent lymphatic glands are moderately enlarged and show no caseation. Microscopic examination of the thickened pleura shows that it is composed of fibrous tissue with none of the characters of tuberculous new growth. Tuberculosis in the neighborhood of the injected pleural cavity had in large part disappeared.

Series C.—The effect of long-continued injections of leucocytes upon the course of experimental tuberculous pleurisy.

With the information derived from the foregoing experiments, an attempt was made to treat with leucocytic injections animals with experimental tuberculous pleurisy. Seven dogs received half a cubic centimeter of the same suspension of tubercle bacilli and at the end of ten days there was in all of them a well-marked increase of thoracic dullness. Three animals were kept as controls whereas the remaining four received repeated injections of leucocytes.

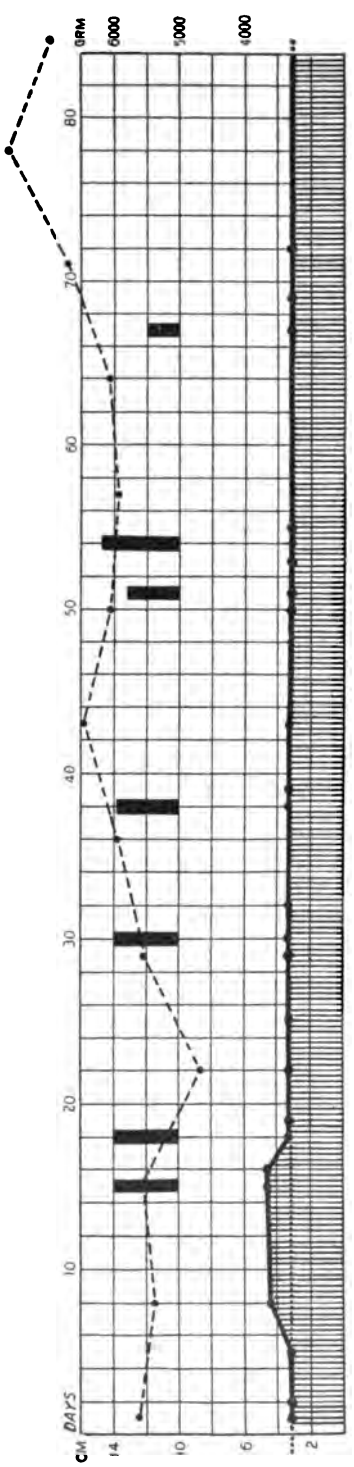


CHART 4. Experiment 11; injection of leucocytes.

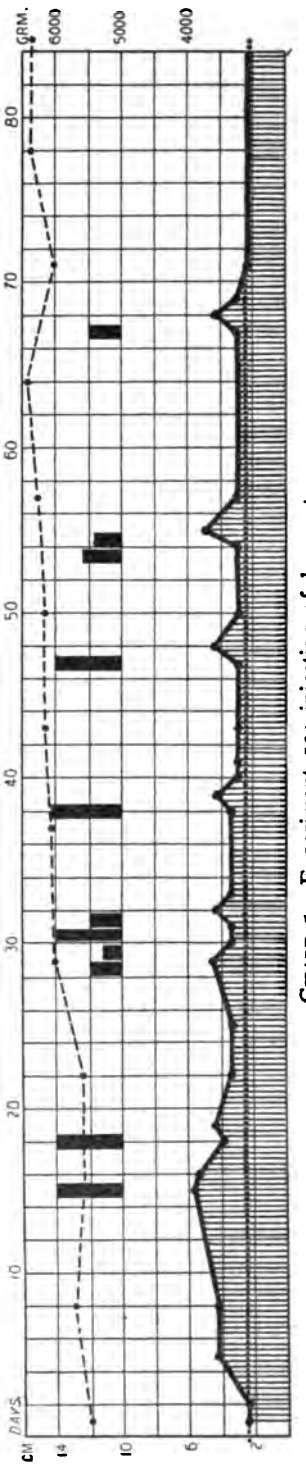
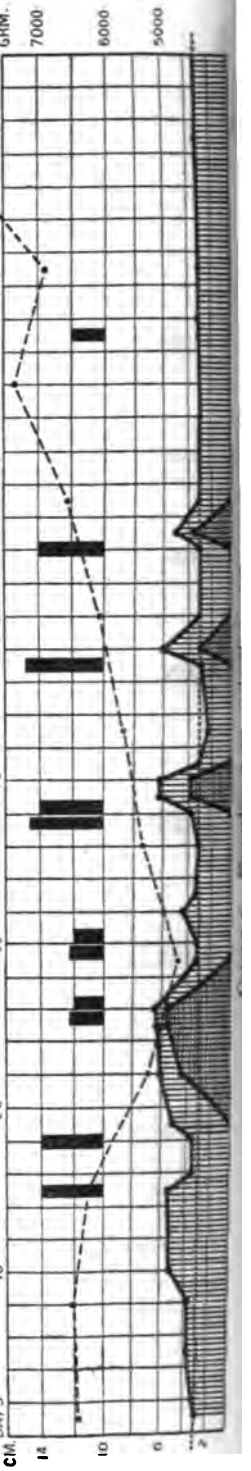


CHART 5. Experiment 12; injection of leucocytes.



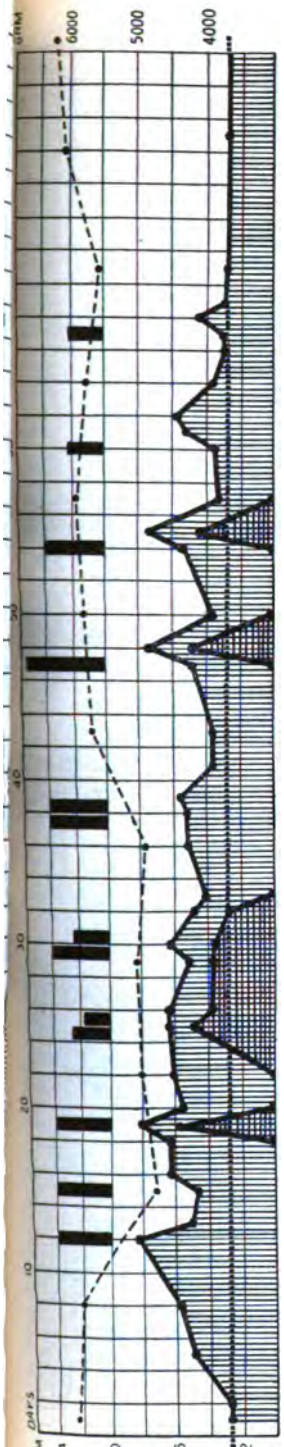


CHART 7. Experiment 14; injection of leucocytes.

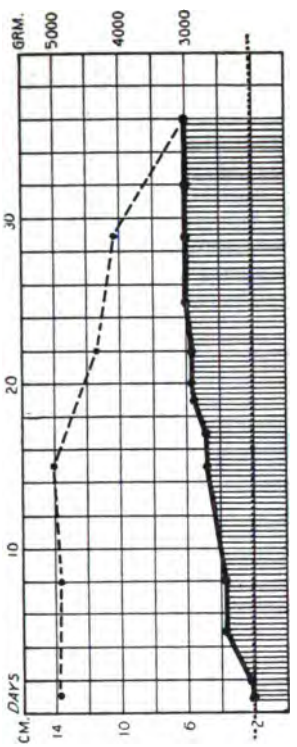


CHART 9. Experiment 16; control.

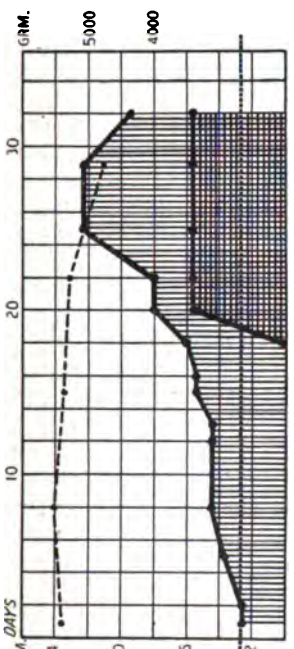


CHART 8. Experiment 15; control.

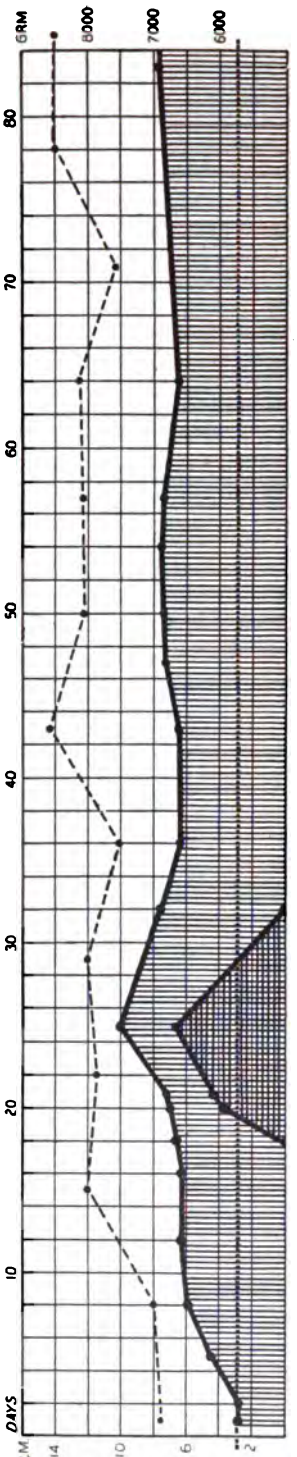


CHART 10. Experiment 17; control.

EXPERIMENT 11.—Dog, wt. 5,650 grm. After inoculation the animal lost about 1,000 grm., the most marked loss in weight following the first two injections of leucocytes. After four weeks the animal regained and subsequently much exceeded its original weight. Thoracic dullness which had increased as the result of inoculation disappeared after the first leucocytic injection and did not reappear (Chart 4). The animal received into the right pleural cavity seven injections. On the twenty-fifth day of the disease a nodule appeared upon the chest wall caused doubtless by infection of the needle tract at the time of inoculation. Leucocytes were twice injected into the nodule which at one time measured 4 cm. across; it diminished in size and almost completely disappeared two weeks after its appearance. The nodule reappeared at the same site and after two weeks was 2 cm. across; four injections of leucocytes (1 c.c.) were not followed by any diminution of size and after attaining a diameter of 4 cm. the nodule broke and discharged during several weeks upon the surface. Healing with scar formation followed. The animal increased much in weight and became strong and active. It acquired the habit of bounding to the top of its cage and would jump continuously during several hours. Five months after inoculation it suddenly became sick and died two days later. At the time of death it was well nourished, weighing one thousand grams more than at the time of inoculation.

Autopsy.—Fat in the subcutaneous tissue and elsewhere is abundant. The pleural cavities contain no fluid. The mediastinum is delicate and membranous and very redundant so that it can be pouched far to the right or left; the membranes below the pericardium are delicate and exhibit the same redundancy. In these membranes and upon the surface of the diaphragm are small, little elevated, patches with reddish gray color; there is no tuberculous tissue and the pleural surfaces are smooth and glossy. The mediastinal lymphatic glands are slightly enlarged, measuring 1 cm. in length and are red and succulent.

The liver is apparently normal. The duodenum near the stomach for a distance of about 7 cm. is plum-colored and apparently in part gangrenous; the adjacent mesentery including the entire pancreas with the exception of a small part of the duodenal arm is infiltrated with blood; the adjacent lymphatic glands particularly those near the liver are enlarged and hemorrhagic. There is no fat-necrosis.

EXPERIMENT 12.—Dog, wt. 5,530 grm. Following inoculation there was slight gradual increase of weight. Thoracic dullness steadily increased during two weeks and diminished immediately after the first injection of leucocytes. During the eleven days between the second and third injection there was increase of dullness which again fell to a level little above that before inoculation. Subsequent elevations above this level were in every instance the result of injection into one or both pleural cavities. In Chart 5 and in subsequent charts injection into both pleural cavities is indicated by two heavy lines side by side, the length of these lines representing the quantity of leucocytes employed. One week after inoculation a nodule appeared in the chest wall at the site of puncture; during ten weeks the nodule received ten injections of leucocytes (0.5 to 1 c.c.). During this time it increased to a maximum diameter of 4 cm. and gradually disappeared without discharging upon the surface. After the fourth intrapleural injection there was cough; an area of relative dullness 5 cm. across

with a peculiar hard resistent character on percussion appeared about the site of injection and persisted several days; cough disappeared. The animal five months after inoculation is well and weighs 1,120 grams more than before inoculation.

EXPERIMENT 13.—Dog, wt. 6,450 gm. After inoculation thoracic dullness gradually increased but its level rapidly fell immediately after the first injection of leucocytes (Chart 6); it rose to a high level after the second injection and absolute dullness appeared but it fell to normal after the third injection which was made into both pleural cavities. At this time the animal was very sick and there was cough and purulent discharge from the nose and eyes; body weight had diminished 1,700 gm. and the animal was very thin. After the fourth week weight steadily increased and evidences of bronchitis disappeared. After the effect of the fourth injection had disappeared there was (on the thirty-eighth day) an increase of thoracic dullness but subsequent elevations (see chart) occurred only as the immediate result of leucocytic injections.

Three weeks after inoculation induration appeared along the tract marked by the inoculating needle; this nodule received one injection of leucocytes (0.5 c.c.) and lying immediately below the skin broke upon the surface. Two subsequent injections were made but it persisted until the seventeenth week after inoculation, disappearing finally.

The animal is large and strong and having grown considerably, its weight is 4,500 grm. greater than before inoculation.

EXPERIMENT 14.—Dog, wt. 6,150 grm. After inoculation thoracic dullness rose quickly to a high level (Chart 7), but fell considerably immediately after the first injection of leucocytes. Body weight diminished rapidly and the animal became very thin. After the second leucocytic injection the level of dullness increased and absolute dullness appeared. Changes following the six subsequent injections, of which with three there was injection into both the right and left pleural cavities, were almost constant, namely, fall of the level of dullness, either immediately or after a preliminary increase, to a level below that at the time of injection and subsequently, after the effect of the injection had disappeared, an increase of dullness. Between succeeding injections the level of dullness became gradually lower and after the immediate effect of the ninth injection had subsided there was no elevation, subsequent increase of dullness occurring only as a sequence of leucocytic injection. After the fifth week of the disease the animal began to gain weight.

A nodule which developed after two weeks at the site of inoculation attained a diameter of 2 cm.; after injection of 0.5 c.c. of leucocytes there was no increase of size. A second injection was made. The nodule diminished in size and during the sixth week of the disease was reresented only by induration at its former site; a third injection was made in the neighborhood of the indurated tissue.

After the second intrapleural injection of leucocytes extensive emphysema of the subcutaneous tissue on the right side of the body made its appearance and disappeared after four days.

The animal is well and strong and weighs 400 grm. more than at the time of inoculation.

Control Experiments.

EXPERIMENT 15.—Dog, wt. 5,300 grm. Control. Thoracic dullness increased gradually during the first three weeks after inoculation and subsequently more rapidly. The animal became thin and died at the end of 35 days. The fall of dullness (and small amount of effusion found at autopsy) suggest that there was rapid absorption of fluid just before death (Chart 8).

Autopsy.—The right pleural cavity contains 25 c.c. of reddish serous fluid; the left cavity contains 5 c.c. Throughout the mediastinum are masses of hard caseous tissue, the largest being situated just above the diaphragm; similar masses occur in the subpericardial membranes on either side. Flat, gray white nodules occur upon the posterior surface of the right lung. The middle lobe of the right lung exhibits pneumonic consolidation. The substernal lymphatic glands are greatly enlarged and firmly caseous; enlarged hard glands are found near the pancreas and in the retroperitoneal tissue beside the adrenal glands.

EXPERIMENT 16.—Dog, wt. 4,950 grm. Control. Thoracic dullness gradually increased from the time of inoculation until death (Chart 9). At the end of about three weeks the animal was very thin and there was abundant purulent discharge from the nose and eyes; an ulcer formed upon the right cornea. Death occurred at the end of 36 days.

Autopsy.—The pleural cavities contain no fluid; the mediastinum is injected and contains small nodules. Above the diaphragm is a caseous mass about 1 cm. across. The substernal lymphatic glands are moderately enlarged and caseous. At the bifurcation of the trachea is a mass of caseous lymphatic glands which encircle and compress the right bronchus. The lungs contain numerous patches of pneumonic consolidation.

EXPERIMENT 17.—Dog, wt. 6,800 grm. Control. After inoculation of the animal, a stout pug, weight rapidly increased and continued much greater than before inoculation. Nevertheless relative dullness increased steadily and absolute dullness made its appearance, disappearing later (Chart 10). Almost immediately after inoculation a nodule appeared at the site of puncture. The nodule increased greatly in size, broke through the skin and discharged during several weeks. The mass below the skin diminished in size and finally disappeared, leaving a small scar. Absolute dullness disappeared at the end of five weeks, but abnormal relative dullness has persisted until the present time. The animal (at the end of five months) is very fat and apparently well, weighing 1,450 grm. more than before inoculation.

Of the animals which were inoculated as controls two died at the end of five weeks, a time corresponding to the time of death of the controls of Series A. The third animal used as control, a relatively large dog, was little affected by the pleural and subcutaneous tuberculosis with which it was infected, but, on the contrary, increased considerably in weight; nevertheless increased thoracic dullness did not return to normal.

Of four tuberculous animals injected with leucocytes one (Experiment 11) exhibited normal thoracic dullness after the third

week and a second (Experiment 12) after the sixth week. In two animals treated with leucocytes the disease was much more severe and there was great loss of body weight. One animal (Experiment 13) passed through a stage in which there were physical signs of fluid in considerable amount, and exhibited normal thoracic dullness only after the sixth week. In the remaining animal (Experiment 14) there was, after the effect of each injection had subsided, an increase of dullness, doubtless referable to the tuberculous process which was still active. Each injection during this period after a primary rise depressed thoracic dullness to a somewhat lower level so that after the seventh week there was no increase except as the result of injection of leucocytes.

In this series of experiments leucocytic injections were at first made at short intervals in relatively small quantity—approximately ten cubic centimeters. Even after thoracic dullness had returned to a level approaching that before inoculation with tuberculosis, leucocytic injections were continued. Since numerous examinations had shown that the tuberculous lesion was bilateral, injections were often made simultaneously into the two cavities.

Series D.—The effect upon thoracic dullness of tubercle bacilli and leucocytes injected simultaneously; the effect of leucocytes preserved during several days at low temperature.

Since the previous experiments have afforded evidence that leucocytes injected into the plural cavity already infected with tuberculosis retard the development of the lesion and tend to cause its disappearance, the possibility has suggested itself that injection of leucocytes, together with tubercle bacilli, might prevent the onset of tuberculosis. The clinical course of the disease in two animals immediately after injection of a mixture of ten cubic centimeters of leucocytes with half of a cubic centimeter of a suspension of tubercle bacilli gave some support to belief that the organism had been wholly destroyed; nevertheless at the end of two weeks such well-marked increase of thoracic dullness occurred that there was no doubt that tuberculosis had developed. The animals were subsequently used to test the efficiency of cells which had been preserved from twenty-four to forty-eight hours at a low temperature, several degrees above the freezing point. One of the animals which

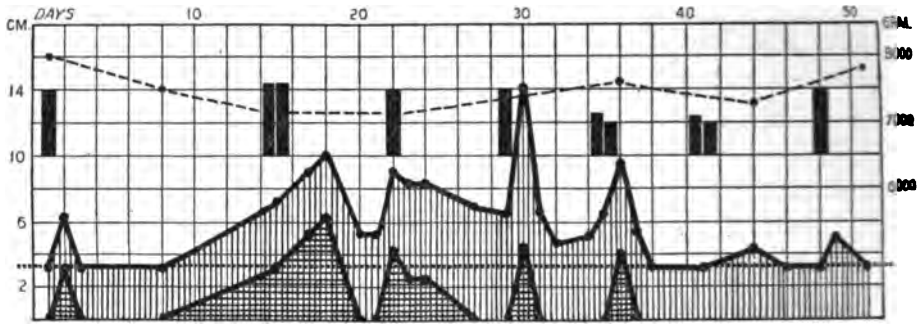


CHART 11. Experiment 18; inoculation with B. tuberculosis and leucocytes; injection of leucocytes.

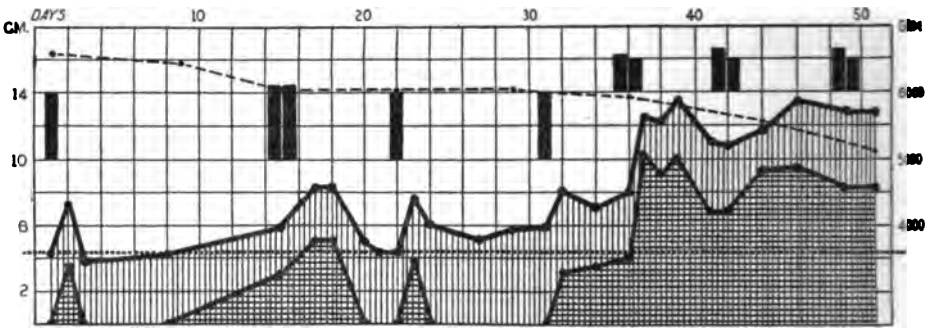


CHART 12. Experiment 19; inoculation with B. tuberculosis and leucocytes; injection of leucocytes preserved at low temperature.

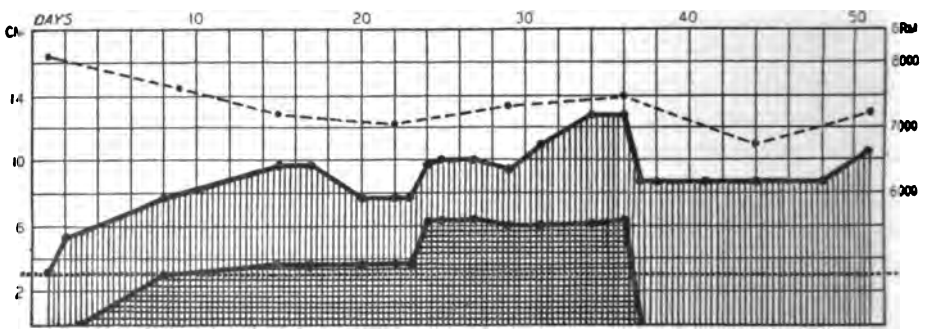


CHART 13. Experiment 20; control.

had received tubercle bacilli and leucocytes was subsequently injected with leucocytes freshly obtained, whereas the second animal which had received the same mixture was injected with leucocytes which had been preserved in cold storage. In some instances the cells employed for corresponding injections in the two animals were identical, the animal in Experiment 19 receiving its injection one or two days after that of Experiment 18. Experiment 20, in which tubercle bacilli alone had been employed, served as control.

EXPERIMENT 18.—Dog, wt. 7,950 grm. The clinical course after inoculation with a mixture of tubercle bacilli and leucocytes is shown in Chart 11. The animal was given after the inoculating injection six injections of freshly obtained leucocytes and was killed at the end of 50 days; it was strong and active when killed.

Autopsy.—The pleural cavities contain no fluid. Several flat fibroid patches occur upon the surface of the lungs, but the pleural surfaces are almost free from evidence of tuberculosis. The subpericardial membranes and mediastinum in front of the heart are delicate save for the presence of a thin firm mass of tuberculous tissue about 7 mm. across. The mediastinum above the level of the heart is thickened and contains several indurated nodules. The substernal lymphatic glands are soft, moderately enlarged, and contain several caseous foci. In the lungs are miliary tubercles. The liver contains numerous small tubercles of which many have a delicate fibrous capsule. In the kidney are a few opaque tubercles.

EXPERIMENT 19.—Dog, wt. 6,700 grm. The clinical course after inoculation, identical with that of Experiment 18, is shown in Chart 12. A nodule appeared at the site of inoculation and after receiving four injections (0.5 to 1 c.c.) of leucocytes kept at low temperature almost completely disappeared, but although injected three times subsequently increased to a diameter of nearly 4 cm. The animal received into the pleural cavities six injections of leucocytes which had been preserved in cold storage during one or two days. The animal which was very weak was killed after 50 days for comparison with the preceding.

Autopsy.—The subcutaneous tissue is jaundiced. The pleural cavities each contain 200 c.c. of fluid. Upon the pleural surfaces are elevated plaques of tuberculous tissue. The mediastinum from the lymphatic glands at the base of the neck, which are enlarged to a diameter of 3 cm., to diaphragm is converted into a thick crinkled mass by partly caseous tissue. Similar masses of large size occupy the subpericardial membranes and are scattered over the right parietal pleura and surface of the diaphragm. In the substance of the right lung corresponding to the site of inoculation is a tuberculous mass; the bronchial lymphatic glands on the same side are much enlarged and tuberculous. The lungs contain tubercles. The liver is enlarged and beset with numerous large tubercles, occupying on section at least a third of the tissue.

Control Experiment.

EXPERIMENT 20.—Dog, wt. 8,050 grm. Control. The animal received 0.5 c.c. of the suspension of tubercle bacilli employed in the two preceding experiments mixed with 10 c.c. of 0.85 per cent. sodium chloride solution. The clinical course is shown in Chart 13. The animal was killed after 50 days.

Autopsy.—The right pleural cavity contains 2 c.c. of red turbid fluid; the left cavity contains 15 c.c. Upon the surfaces of the lungs and upon the diaphragm are numerous flat tubercles. The mediastinum and subpericardial membranes are thickened and beset with numerous tubercles. A hard mass of gray white tissue (1.2 cm. across) is situated in the right, a second (2 cm. across) in the left subpericardial membrane at its junction with the diaphragm; a third mass is situated in the mediastinum above the diaphragm. The substernal lymphatic glands are moderately enlarged, hard and almost wholly caseous. In the lungs are miliary tubercles. The liver contains numerous tubercles; in the kidney opaque tubercles often 1.5 cm. in diameter are fairly numerous.

In the animal of Experiment 20, used as control, relative and absolute thoracic dullness increased gradually after inoculation, but diminished suddenly after the thirty-fifth day; nevertheless relative dullness maintained a high level until the animal was killed. In the dog of Experiment 18, which received fresh leucocytes, the clinical course was identical with that illustrated by Experiments 11 to 14 of Series C, namely, depression of dullness, perhaps preceded by temporary increase, after each injection. Comparison of these two animals of the present series confirms the result of former experiments and affords clinical and anatomical evidence that the presence of artificially introduced leucocytes has retarded the development of the tuberculous lesion.

The employment of leucocytes which have been preserved at a low temperature has not had an equally favorable result, but the experiment is indecisive, for autopsy has shown that the lung has been punctured at the time of inoculation. Tuberculosis of the lung and of the bronchial glands doubtless explains in part the rapid progress of the disease. Nevertheless the charted thoracic dullness shows that changes which follow injection of leucocytes kept at low temperature during several days may be identical with those caused by freshly obtained leucocytes. Injection on the fifteenth day of the disease was followed after an interval of several days by diminution of relative dullness and disappearance of absolute dullness referable to diminution of the fluid contents of the chest.

Series E.—The clinical and pathological changes following simultaneous injection of tubercle bacilli and leucocytes.—Injection of leucocytes together with tubercle bacilli in the preceding experiments has been followed by the reaction which occurs when leucocytes are injected into the normal pleural cavity (see Charts 11 and 12); there is accumulation of fluid which quickly disappears. Subsequent increase of dullness, which is the otherwise constant result of tuberculous pleurisy, is delayed. The same experiment has been repeated and, in order that the resulting changes may be compared by anatomical examination, the animals have been killed as soon as that which has received leucocytes has exhibited increase of thoracic dullness.

EXPERIMENT 21.—Dog, wt. 5,950 grm. A suspension of tubercle bacilli (0.5 c.c.) mixed with 10 c.c. of leucocytes which had been kept at a temperature slightly above freezing during three days was injected into the right pleural cavity. The level of thoracic dullness (Chart 14) rose abruptly, subsided, and remained normal during at least a week; it then rose and the animal was killed 16 days after inoculation.

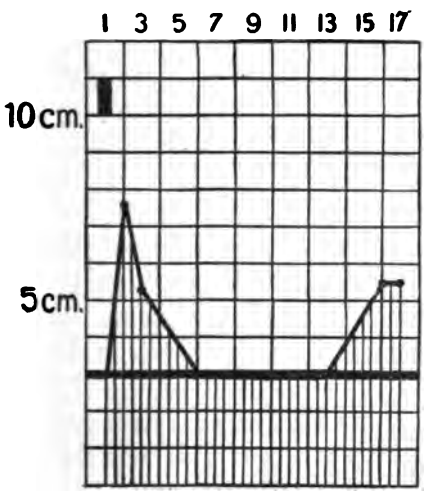


CHART 14. Experiment 21; inoculation with B. tuberculosis + leucocytes.

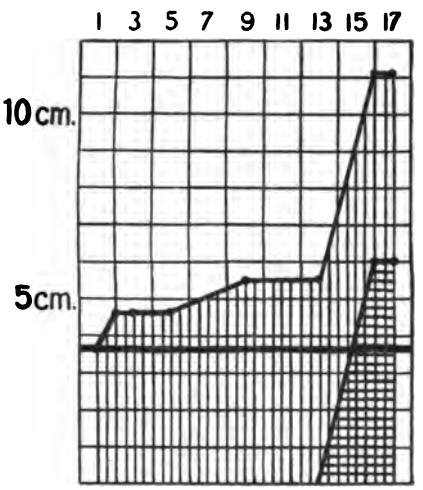


CHART 15. Experiment 22; control.

Autopsy.—The right pleural cavity contains a small amount of turbid whitish fluid (not measured); the left cavity contains a somewhat greater quantity. The mediastinum is delicate and contains a few tubercles; the right and left subperi-

cardial membranes are studded with tubercles. Just above the diaphragm is a small, firm, succulent mass. The mediastinal lymphatic glands are moderately enlarged, measuring 1.2 cm. The surfaces of the diaphragm and the parietal pleuræ are smooth. The liver contains small miliary tubercles.

EXPERIMENT 22.—Dog, wt. 6,900 grm. Control. A suspension of tubercle bacilli (0.5 c.c.) was diluted with physiological salt solution to 10 c.c. and injected into the right pleural cavity. Thoracic dullness (Chart 15) increased gradually during 12 days and then more rapidly. The animal was killed at the end of 16 days.

Autopsy.—The right pleural cavity contains 120 c.c. turbid, whitish, coagulable fluid; the left cavity, 95 c.c. Upon the surface of the diaphragm and upon the parietal pleura in greatest abundance on the right side are large, flat, gray white nodules. The mediastinum is thick and beset with small tubercles and partially caseous masses, the largest being just above the diaphragm; the subpericardial membranes on either side contain large hard masses of gray white tissue. The substernal lymphatic glands are considerably enlarged, measuring 1.7 cm. The liver contains small miliary tubercles.

The changes of thoracic dullness observed in Experiment 21 after inoculation with teubercle bacilli mixed with leucocytes are identical with those noted immediately after injection of the same mixture in two animals of the preceding series; in each series there has been the same contrast with the control. The persistence of normal thoracic dullness during a week after the preliminary rise and fall suggests that the development of the tuberculous process has been retarded. Twenty-four hours after the appearance of abnormal dullness in the animal which received leucocytes both animals of the present series were killed; effusion was abundant in the control but much less in the animal with leucocytes; tuberculous pleural nodules were numerous and massive in the former, but almost absent in the latter; the membranes within the thorax contained abundant tuberculous tissue in the one, but little in the other. The substernal lymphatic glands were much larger in the control.

The experiment furnishes additional evidence that leucocytes tend to check the development of a tuberculous lesion, even though they have been preserved during several days at a temperature a little above the freezing point.

Series F.—Anatomical changes found in animals with tuberculous pleurisy killed after repeated injections of leucocytes.

In the following series of experiments both control and animal treated by intrapleural injections of leucocytes were killed at the

same interval after inoculation in order to determine by anatomical examination the effect of the injected cells. The animals in the two following experiments were of unequal size, that which received injections being a small puppy; each received into the right pleural cavity half of a cubic centimeter of the same suspension of tubercle

EXPERIMENT 23.—Dog, wt. 4,150 gm. During the first two weeks after inoculation relative thoracic dullness over the infected cavity increased from 3 to 7.7 cm. Injection of leucocytes (16 c.c.) on the fourteenth day was followed by a fall of this level. Two subsequent injections caused diminution of the area of dullness which after the third week twice returned to normal but rose slightly after the effect of the injection had disappeared. Twenty-four hours before the animal was killed relative dullness measured 4.4 cm. and the animal received into the right pleura 9.5 c.c. leucocytes. Cough and purulent nasal discharge appeared during the third week of the disease and persisted until death. There was a nodule at the site of inoculation which received one injection of leucocytes; it did not increase in size but remained until death. The animal was killed after 32 days.

Autopsy.—The right pleural cavity contains 12 c.c. turbid blood-stained fluid; the left, 11 c.c. The parietal pleura is smooth save at one point corresponding to the site of inoculation where there is a group of small nodules; upon the opposing surface of the lung and extending into the substance in an area of fibrous tuberculous tissue. The bronchial lymphatic glands on the right side are enlarged and tuberculous. In the mediastinum and subpericardial membranes are tuberculous masses of considerable size. The substernal lymphatic glands are greatly enlarged and caseous. Tuberculous glands are found near the pancreas. The lungs contain patches of broncho-pneumonia and miliary tubercles.

EXPERIMENT 24.—Dog, wt. 7,000 gm. Control. Two weeks after inoculation relative dullness had increased from 3 to 8.6 cm. and absolute dullness had made its appearance; on the eighteenth day relative dullness was 12 cm. and absolute dullness 8.5 cm. These levels were maintained until the animal was killed 32 days after inoculation. A nodule appeared at the site of inoculation and broke upon the surface of the skin.

Autopsy.—The right pleural cavity contains 235 c.c., the left 184 c.c. of turbid pale yellow fluid. The pleural surfaces are intensely injected and the parietal pleura is thickly beset with flat tuberculous nodules which near the diaphragm are confluent. The mediastinum is occupied by a large mass of hard tuberculous tissue extending into the left subpericardial membrane. The substernal lymphatic glands are greatly enlarged and caseous; tuberculous glands occur near the pancreas. The lungs contain miliary tubercles.

Injection of leucocytes was followed by diminution of thoracic dullness already increased by tuberculous pleurisy but even before the animal was killed it was evident that the tuberculous process was still active, for slight increase of dullness had occurred just before death. Difference between injected and uninjected animals

was well marked in the pleural cavities; in the control there was fluid in great quantity, the pleural membranes were injected and raised tuberculous plaques were present in immense numbers upon the parietal pleura, whereas in the injected animal the pleural surfaces were smooth and the cavities contained very little fluid. Elsewhere, both in the control and in the injected animal, tuberculosis was advanced and widely disseminated. It is noteworthy that the lung had been punctured at the time of inoculation, so that, although as the subsequent course of the disease showed, the pleura had been infected, there was tuberculosis of the lung and of the bronchial lymphatic glands as well.

The following experiments are described to illustrate further the anatomical effect of injected leucocytes. They confirm those which have already been cited and furnish additional evidence that injected leucocytes influence in greatest degree tuberculosis in structures with which they come into immediate contact. One animal, used as control, received a suspension of tubercle bacilli (0.5 c.c.). The other animal received the same suspension (0.5 c.c.) mixed with leucocytes (10 c.c.); subsequently leucocytes were injected into one or both pleural cavities at intervals of about a week.

EXPERIMENT 25.—Puppy, wt. 5,650 grm. After simultaneous injection of leucocytes and tubercle bacilli there was no permanent increase of thoracic dullness until the sixth day when it had increased from 3.2 to 3.7 cm. Subsequent injections of leucocytes into one or both pleural cavities reduced this level and it remained at the normal level save as the immediate result of injection until death. There was bronchitis and the animal lost weight. The animal was killed after 33 days.

Autopsy.—The pleural cavities contain no fluid and there is no tuberculous tissue in the adjacent membranes. The mediastinum and subpericardial membranes are delicate and evidently greatly stretched so that they form redundant folds and can be pouched far to the right or left (Fig. 4). The parietal and visceral pleurae are smooth except for patches and shreds of soft reddish tissue which represent perhaps the site of tuberculous plaques. The substernal lymphatic glands are enormously enlarged, measuring 3.5 cm. in length; they are homogeneously caseous and surrounded by a fibrous capsule. The lungs contain an occasional miliary tubercle; the liver contains innumerable tubercles.

EXPERIMENT 26.—Puppy, wt. 6,050 grm. Control. Thoracic dullness, measuring before inoculation 2.8 cm., increased continuously after inoculation; there was cough and purulent discharge from the nose after the third week. A tuberculous nodule formed at the site of inoculation. At the end of 32 days relative dullness had increased to 8.2 cm., absolute dullness to 4.6 cm. The animal was killed at the end of 33 days (24 hours before death 10 c.c. of leucocytes had been injected into the right pleural cavity).

Autopsy.—The right pleural cavity contains 170 c.c. of turbid fluid; the left, 125 c.c. of less turbid coagulable fluid. The parietal pleura is injected and rows of tuberculous plaques are situated between the ribs; similar plaques occur upon the diaphragm. The mediastinum above the diaphragm contains a tuberculous mass of great size extending into both subpericardial membranes which are thickened and studded with tubercles (Fig. 5). The mediastinum above this mass is thickened and beset with tubercles and larger tuberculous masses. The substernal lymphatic glands are enormously enlarged, 3.5 cm. in length and caseous. Tubercles are moderately numerous in the lungs and are present in enormous number in the liver.

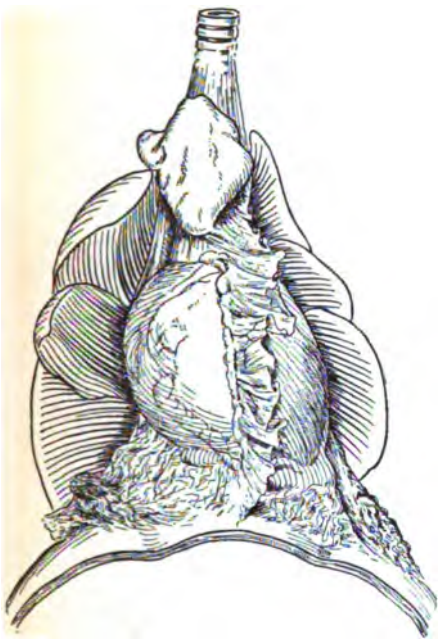


FIG. 4. Experiment 25; animal injected with leucocytes.

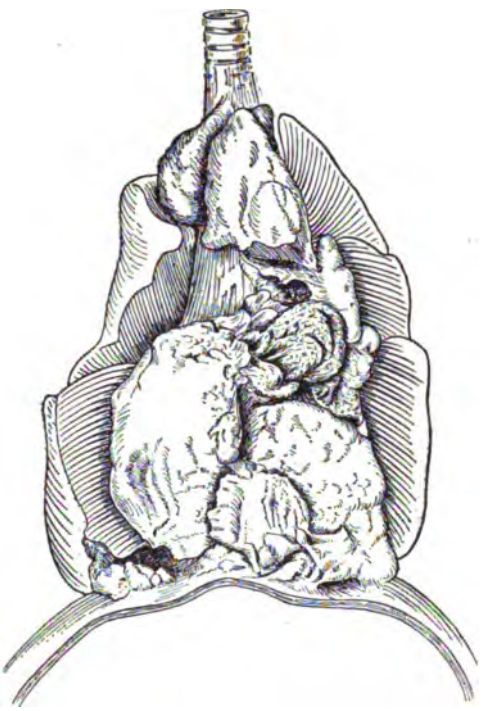


FIG. 5. Experiment 26; control.

In the animal which had received leucocytes the pleural membranes exhibited no evidence of tuberculosis whereas in the control there was pleural effusion and advanced tuberculosis of the pleura and mediastinum (compare Figs. 4 and 5). Nevertheless tubercle bacilli had found lodgment in the substernal lymphatic glands of the injected animal; tubercle bacilli perhaps disseminated from this focus had caused miliary tuberculosis of lungs and liver.

In the following experiment repeated injection of leucocytes had little effect upon the development of tuberculosis.

EXPERIMENT 27.—Dog, wt. 4,850 grm. On the fifth day after simultaneous injection of tubercle bacilli (0.5 c.c.) and leucocytes thoracic dullness had increased to 4.3 cm., the original level being 2.5 cm. Injection of leucocytes repeatedly reduced thoracic dullness but failed to restore it to normal. Cough appeared and was accompanied by purulent nasal discharge, conjunctivitis and ulceration of the right cornea. The animal lost weight and became sick and weak. It was killed after 39 days; just before death relative dullness measured 5.5 cm., absolute dullness 2.8 cm.

Autopsy.—The right pleural cavity contains 5.5 c.c. of almost clear fluid; the left cavity contains 10 c.c. of very turbid yellowish fluid. Upon the parietal pleura and diaphragm is an occasional tuberculous plaque. The mediastinum from substernal glands which are greatly enlarged and caseous to diaphragm is occupied by yellowish white tissue which is partly caseous and partly fibrous. Almost the entire right lung is consolidated by mottled red and gray patches of broncho-pneumonia; the left lung contains similar patches. The liver contains tubercles; lymphatic glands near the pylorus are enlarged and caseous.

EXPERIMENT 28.—Dog, wt. 4,400 grm. Control. Thoracic dullness (normal relative dullness measured 3.2 cm.) increased continuously after inoculation with 0.5 c.c. suspension of B. tuberculosis mixed with 10 c.c. salt solution; a nodule appeared at the site of inoculation. There was cough which disappeared. The animal was killed at the end of 39 days; before death relative dullness measured 8.5 cm. and absolute dullness 4.6 cm.

Autopsy.—The right pleural cavity contains 39 c.c. of opaque fluid; the left cavity, 28 c.c. The parietal pleura is intensely injected and upon its surface are flat inconspicuous tuberculous nodules. In the mediastinum above the diaphragm extending far into the subpericardial membranes on each side is a very large firm tuberculous mass of pearly white color. The substernal lymphatic glands are greatly enlarged and continuous with a large tuberculous mass in the mediastinum situated immediately below them. The lungs contain a few scattered tubercles; tubercles are numerous in the liver.

Series G.—Experiments with a more virulent tubercle bacillus.—The foregoing experiments have been performed with a strain of tubercle bacillus of moderate virulence; this organism killed guinea-pigs in three or four weeks after intraperitoneal inoculation, but failed to kill rabbits when injected into the peritoneal cavity. In the following experiments a much more virulent organism which killed rabbits five weeks after intraperitoneal inoculation was used. Its virulence was well illustrated by the effect on dogs—death occurred with considerable rapidity, and instead of the gray-white sarcoma-like masses containing foci of caseation new formed tissue, which exhibited almost homogeneous caseation, was the result of fatal inoculation.

EXPERIMENT 29.—Dog, wt. 6,050 grm. Dullness over the right side of the chest increased during the week following inoculation and was reduced by the injection of leucocytes (Chart 16). The second injection produced no decrease, but the third caused material fall of the levels of relative and absolute dullness. Subsequent injections were not equally favorable. A nodule appeared in the chest wall at the site of inoculation and was injected four times with leucocytes (0.5 c.c.); it increased in size measuring 4.5 cm. across, and then diminished slightly. The animal became thin and weak and died after 39 days.

Autopsy.—The right pleural cavity contains 60 c.c. of serous slightly turbid fluid; the left cavity contains 35 c.c. of less turbid fluid. The pleural membranes are intensely injected. The mediastinum contains a flat mass of greenish caseous material (Fig. 6); a second mass just above the diaphragm extends into the left subpericardial membrane; the corresponding membrane on the right side is uniformly thickened by partly caseous tissue. The mediastinal lymphatic glands are enlarged and caseous. Distributed over the pericardium diaphragm and pulmonary surfaces are plaques of yellow tissue. The chest wall (at the site of inoculation) contains an ill-defined area of thickening and caseation and opposite in the substance of the lung is a round nodule of tuberculous tissue; the bronchial lymphatic glands on the right side are tuberculous. The lungs contain a few small miliary tubercles. The liver is enlarged and jaundiced and contains tubercles in immense number.

EXPERIMENT 30.—Dog, wt. 7,500 grm. Control. After inoculation thoracic dullness increased gradually (Chart 17); a small nodule formed in the skin at the site of inoculation. The animal died at the end of 47 days.

Autopsy.—The right pleural cavity contains 195 c.c. of yellow opaque fluid; the left, 150 c.c. The pleural membranes are injected more markedly on the right side; on the parietal pleura of the right side are flat yellow tubercles. The entire mediastinum is occupied by a greenish-yellow somewhat soft caseous mass merging into the greatly enlarged caseous substernal lymphatic glands. Both subpericardial membranes are greatly injected and contain large caseous masses (Fig. 7). Opposite the site of inoculation is a small caseous nodule 0.5 cm. across and extending about 1.5 mm. into the substance of the lung. The lungs contain no tubercles; in the liver are numerous miliary tubercles.

Tuberculosis was almost equally advanced in the two animals, although in that which received leucocytes tuberculous masses in the mediastinum and elsewhere were smaller. Wide dissemination in the injected animal is referable in part to inoculation of the lung. In the following experiment both injected animals and control were killed at the end of the same period and neither gave evidence that the lung substance had been entered by the tubercle bacilli with which they had been inoculated.

EXPERIMENT 31.—Dog, wt. 5,250 grm. A gradual increase of dullness followed inoculation (Chart 18); the first injection caused a rapid increase of dullness followed by decrease maintained until the next injection. This injection was followed by an increase which did not subside. There was accumu-

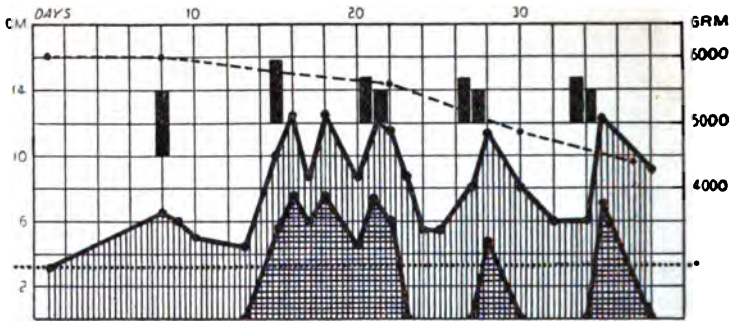


CHART 16. Experiment 29; injection of leucocytes.

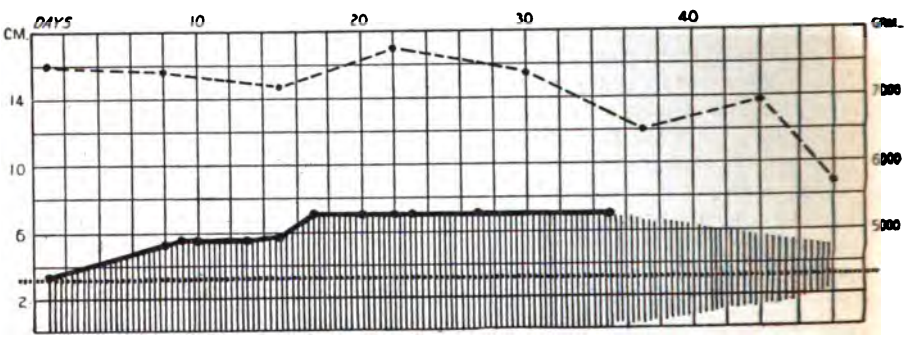


CHART 17. Experiment 30; control.

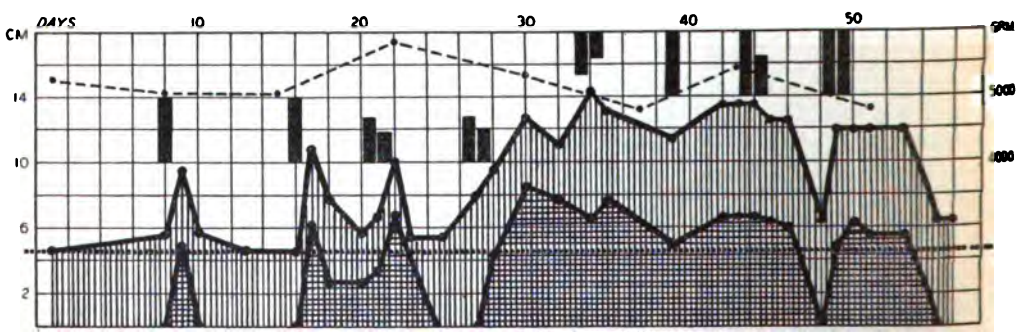


CHART 18. Experiment 31; injection of leucocytes.

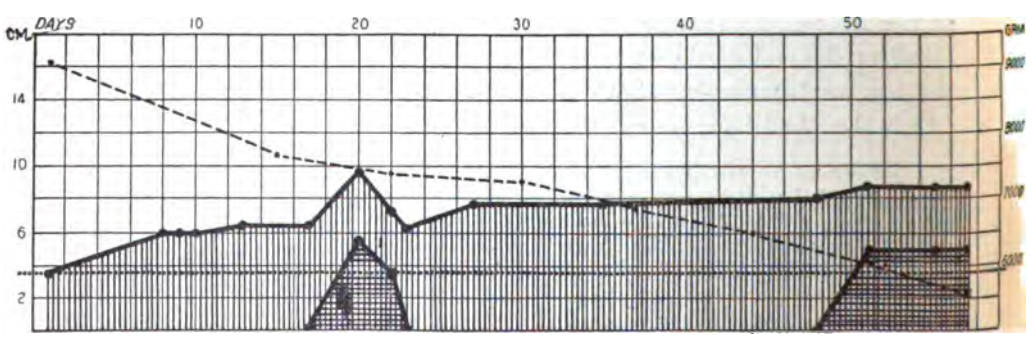


CHART 19. Experiment 32; control.

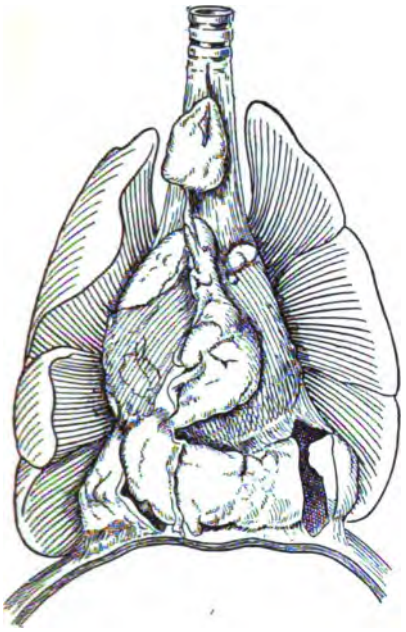


FIG. 6. Experiment 29; animal injected with leucocytes.

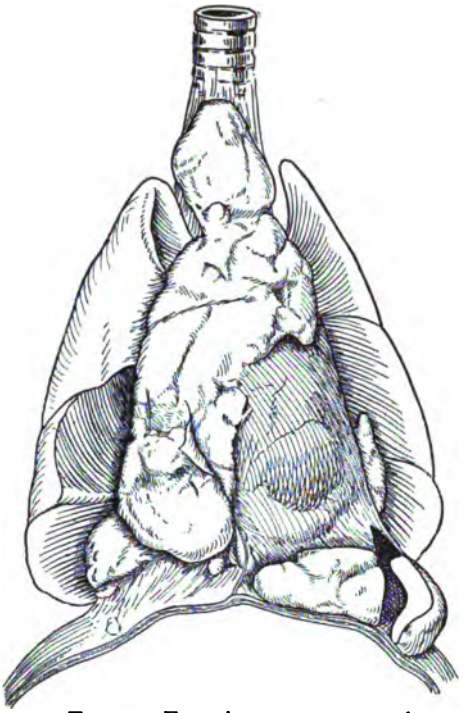


FIG. 7. Experiment 30; control.

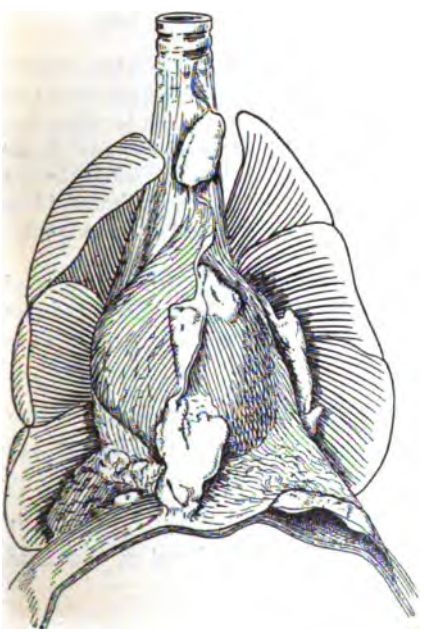


FIG. 8. Experiment 31; animal injected with leucocytes.

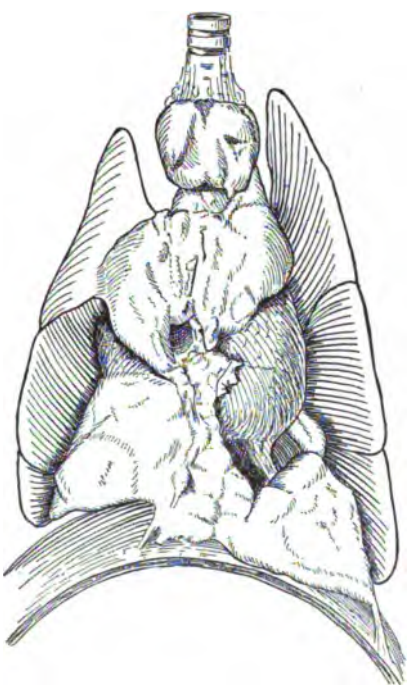


FIG. 9. Experiment 32; control.

lation of a large pleural effusion and subsequent injections exhibited only occasional tendency to depress its level (fifth and seventh injection). A nodule formed in the chest wall during the third week after inoculation, increased in size and broke upon the surface; it was injected three times with leucocytes. During the seventh week of the disease a swelling appeared over the lower bone of the right hind leg and soon breaking upon the surface formed an ulcer with undermined edges about which there was redness of the skin. Leucocytes similar to those previously employed were applied to the wound and injected beneath its edges; a sterile dressing was put about the leg. Two days later when the bandage was removed swelling and redness had almost disappeared and the ulcer was clean and dry but exhibited no tendency to heal. The animal became thin and very weak and died 55 days after inoculation.

Autopsy.—The right pleural cavity contains 33 c.c. of deep yellow fluid; the left cavity, 14 c.c. of reddish fluid. The pleura of the diaphragm and chest wall is roughened by a thin irregularly distributed layer of fibrin and on both parietal and pulmonary pleuræ occur a few small tuberculous plaques. The mediastinum is thickened and contains small masses of friable caseous tissue from 1 to 3 mm. in thickness (Fig. 8). Similar thin plaques of caseous material with rough surface of somewhat eroded appearance occupy the subpericardial membranes. The substernal lymphatic glands are hard and caseous, 1.7 cm. in length. The lungs contain no tubercles recognizable by macroscopic examination. The liver is enlarged and contains tubercles. The spleen is large and soft, but exhibits no tubercles. Lymphatic glands in contact with the pancreas are enlarged and caseous; the mesenteric glands show the same alteration.

EXPERIMENT 32.—Dog, wt. 9,050 grm. Control. After inoculation thoracic dullness increased during three weeks (Chart 19), decreased and again increased; a nodule formed at the site of inoculation. The animal became thin and weak and conjunctival jaundice was present before it was killed, when almost moribund, 55 days after inoculation.

Autopsy.—The right pleural cavity contains 104 c.c. of almost clear, amber yellow fluid; the left pleural cavity, 116 c.c. The pleura is slightly thickened and upon the parietal and visceral surfaces, particularly on the right side, are numerous flat nodules. The mediastinum is replaced by a great mass of fairly soft, greenish yellow, in great part caseous tissue extending the whole length of the sternum and projecting outward into each subpericardial membrane (Fig. 9). The substernal lymphatic glands are greatly enlarged and caseous, measuring 2.3 cm. in length.

The lung on the surface and in its substance contains an immense number of tubercles. The liver is jaundiced and contains innumerable tubercles. The spleen is enlarged and contains thickly scattered opaque tubercles. Tuberculous glands of great size occur near the pancreas.

These experiments, in which a virulent tubercle bacillus was used, were undertaken under unfavorable conditions; dogs of approximately equal size were not obtainable and the dogs used as controls were much larger (9,050 and 7,500 grm.) than those which were injected (6,050 and 5,250 grm.). Fall of the level of thoracic

dullness frequently followed injection of leucocytes, but was less constant than in preceding experiments in which the less virulent microörganism was employed. It is noteworthy that for two injections in one animal (third and fourth injections in Experiment 29) and for one injection in the other (fourth in Experiment 31) cells were used which were obtained four and five days after injection of turpentine and were almost entirely necrotic. Nevertheless, although the injections failed to prolong life, the anatomical condition observed at autopsy showed that they had exerted an influence upon the development of the tuberculous lesion similar to that which was evident when somewhat less virulent microörganisms had been inoculated.

The quantity of fluid found at autopsy in the pleural cavities of the control animals has been far greater than that of the injected animals. The diagrams which show the size and distribution of tuberculous tissue indicate that the lesion has been more advanced in the controls, which contain throughout the mediastinum large masses of caseous tissue merging into one another (see Figs. 7 and 9). In the injected animals (see Figs. 6 and 8), especially in Experiment 31, tuberculous masses are less extensive and are of smaller size. In Experiment 30 the lung has been punctured and infected at the time of inoculation, so that there is no opportunity of comparing the effect of injections upon general dissemination of tuberculosis, but between Experiment 31 and its control, Experiment 32, in which the animals lived the same length of time, comparison is possible; tuberculosis in the lungs, liver and spleen was more advanced in the control than in the injected animal.

The almost constant effect of leucocytes injected into the pleural cavity of an animal from a week to ten days after intrapleural inoculation with tubercle bacilli is a fall of the level of thoracic dullness, elevated by the presence of effusion or of newly-formed tuberculous tissue. This fact is especially noteworthy because leucocytes in similar amount injected into the normal pleural cavity cause a rapid but temporary accumulation of fluid and increase of dullness over the dependent part of the chest. The first injection of leucocytes into eleven tuberculous animals was followed in seven instances by a fall of dullness recognizable twenty-four hours later;

in two experiments there was no depression of the level of dullness until forty-eight hours later. In only two animals did a reaction occur resembling that of the normal animal, namely, rise of dullness following injection with subsequent fall; in one instance this level finally fell below the level at time of injection, but in one instance it remained above the previous level.

Injections after the first exhibited a similar almost constant tendency to reduce the level of thoracic dullness; a preliminary elevation followed within two or three days by a fall below the level at the time of injection was frequently observed.

The relation of depression of thoracic dullness to diminution of pleural effusion is well shown by the amount of fluid present at autopsy in the chest of injected and uninjected animals. The following figures represent the quantity of fluid present in the chest of such animals dead or killed at approximately equal intervals after inoculation.

Injected Animal.			Control.		
Number.	Right.	Left.	Number.	Right.	Left.
Experiment 18	0 c.c.	0 c.c.	Experiment 20	2 c.c.	15 c.c.
23	12	11	24	235	184
25	0	0	26*	170	125
27	5.5	10	28	39	28
29	60	35	30	195	150
31	33	14	32	104	116

In one series of experiments (Series B) the animal which had received injections contained more effusion than the control; in this instance chronic non-tuberculous pleurisy not apparently referable to the tubercle bacillus had been caused by frequently repeated and very large injections of leucocytes. The experiment was performed at the beginning of the present study and accurate measurement of the quantity of fluid was not made.

Diminution of abnormal thoracic dullness caused by repeated injection of leucocytes into the tuberculous pleural cavity is doubtless due to diminution of tuberculous tissue in and about the mediastinum as well as to diminution of fluid. Evidence of the disappearance of such tissue is obtainable only by autopsy and in a number

* In this experiment leucocytes were injected into the pleural cavity twenty-four hours before death.

Animal Injected with Leucocytes.							Control.						
Number.	Duration of Disease.	Disseminated Tubercles on Pleural Surfaces	Tuberculous Masses in Mediastinum.	Length of Substernal Lymphatic Glands.	Dissemination of Miliary Tubercles Elsewhere.	Remarks.	Number.	Duration of Disease.	Disseminated Tubercles on Pleural Surfaces	Tuberculous Masses in Mediastinum.	Length of Substernal Lymphatic Glands.	Dissemination of Miliary Tubercles Elsewhere.	Remarks.
Exp. 23	32 days	Absent	Large	3.0 cm.	Liver Lungs	Puncture of lung	Exp. 24	32 days	Numerous	Large	3.1 cm.	Liver Lungs	
Exp. 25	33 days	Absent	Absent	3.5 cm.	Liver Lungs		Exp. 26	33 days	Numerous	Large	3.5 cm.	Liver Lungs	
Exp. 27	39 days	Present in small number	Large	2.3 cm.	Liver		Exp. 28	39 days	Present in considerable number	Large	3.0 cm.	Liver Lungs	
Exp. 29	39 days	Numerous	Large	2.0 cm.	Liver Lungs	Puncture of lung	Exp. 30	47 days	Numerous	Large	3.0 cm.	Liver	Puncture of lung
Exp. 18	50 days	Present in small number	Small	1.6 cm.	Liver Lungs Kidneys		Exp. 20	50 days	Numerous	Moderately large	1.2 cm.	Liver Lungs Kidneys	
Exp. 31	55 days	Present in small number	Small	1.7 cm.	Liver		Exp. 32	55 days	Numerous	Large	2.3 cm.	Liver Lungs Spleen	
Exp. 9	56 days	Absent	Small and fibroid	1.4 cm.	Liver Lungs Kidneys		Exp. 10	56 days	Numerous	Large	2.1 cm.	Liver	

of instances comparison has been made between injected and uninjected animals dead or killed at the same interval after inoculation with the same suspension of tubercle bacilli. The attempt has been made to illustrate the effect of leucocytes by the accompanying table in which parallel injected and control animals are compared.

It is noteworthy that in two instances in which the difference between injected and uninjected animals has been comparatively slight (Experiments 23 and 29) the inoculated tubercle bacilli have entered in part the substance of the lung, for opposite the site of injection there have been tuberculous masses within the organ and the bronchial lymphatic glands on the same side have been enlarged and tuberculous. In these cases examination during life has demonstrated the existence of tuberculous pleurisy, but coexisting pulmonary tuberculosis has doubtless formed a second focus from which dissemination might occur, so that the disease perhaps has been less readily influenced by the injected leucocytes.

The influence of injected cells has been found most evident where it may be assumed that the injected material has been in contact with tuberculous tissue. The pleural membranes exhibit in most marked degree the effect of the injected leucocytes. In injected animals the flat plaques of tuberculous tissue which occur in the controls upon the parietal pleura and diaphragm are almost or wholly absent; nodules and larger masses of newly formed tissue in the mediastinum have been much smaller after injections and the membrane has had in Experiments II (not included in the table) and in Experiment 25 the delicate translucent appearance observed in the normal animal. Tuberculous masses situated in the subpericardial membranes on either side are similarly retarded in their development.

The long diameter of the substernal lymphatic glands is given in the table as a crude measure of tuberculosis in these organs. Comparison of injected and uninjected animals killed after the same interval shows that these tuberculous glands have usually attained a greater size in the uninjected than in the injected animals, but this difference is not constant. Cells doubtless influence with greater difficulty these more remote structures and in Experiment 25 the pleural cavities of the injected animal were normal, but the substernal lymphatic glands were greatly enlarged.

The late changes which may occur as the result of injection of leucocytes in large quantity are well illustrated by Experiment 9. The control contained in the mediastinum and adjacent membranes large succulent masses of tuberculous tissue, whereas in corresponding situation in the injected animal there was scar-like fibrous tissue; the substernal lymphatic glands of the control were large, hard and tuberculous, whereas those of the injected animal were only moderately enlarged and did not exhibit evidence of tuberculosis.

Complete restoration to normal is better illustrated by Experiment 11 of Series C; the pleural surfaces are smooth and the mediastinal and subpericardial membranes, though stretched and redundant, are delicate and transparent. Tags of soft reddish tissue represent apparently the site of tuberculous nodules. The condition of the pleural cavities and adjacent membranes is identical in Experiment 25.

Evidence obtained from animals killed at varying intervals after inoculation does not afford evidence that injection of leucocytes increases or diminishes dissemination of tuberculosis. Both in controls and in injected animals the liver is invariably the seat of miliary tubercles, which may be numerous ten days after inoculation. Widespread distribution occurred in the injected animal of Experiment 9 and in the control, Experiment 32, but in each instance was absent in the animal used for comparison. Recovery of animals shows that disappearance of the local lesion tends to limit dissemination of bacteria.

The mechanism of the process by which leucocytes, obtained by use of a sterile irritant, inhibit the development of a tuberculous lesion has not been considered. It is noteworthy that the cells injected consist of polynuclear and in equally great number of mononuclear cells. The histology of tuberculosis in the dog presents features of interest and has exhibited alterations apparently as the result of leucocytic injection. Experiments which have been cited have suggested that leucocytes which are living can alone accomplish the changes which have been described. These details require special study.

THE PATHOLOGICAL ANATOMY OF HYDRAZINE POISONING.[1]

By H. GIDEON WELLS.

(*From the Pathological Laboratory of the University of Chicago.*)

Plate XXVII.

In a recent article in the *Journal of Biological Chemistry*[2] is reported a study of the influence of hydrazine upon the intermediary metabolism in the dog, by Professor F. P. Underhill and I. S. Kleiner, in which it is shown that although this poison produces extensive anatomical alterations in the liver of dogs, yet it causes little change in the partition of urinary nitrogen and sulphur, beyond that due to the starvation from which animals poisoned with this drug suffer as they always refuse food while under the influence of the drug. During these studies tissues from the poisoned dogs were kindly placed at my disposal by Professor Underhill for histological examination, and I also poisoned and examined the tissues of a few other dogs, as well as of cats and guinea pigs. A summary of the results of this histological study has been included in the article mentioned, but it has seemed that the histological changes observed are so remarkable and of such importance that they deserve a somewhat more extensive description and consideration.

Hydrazine, $NH_2 — NH_2$, is not a substance that is likely to become so commonly used that it will be of general toxicological importance, although the phenyl derivative, phenyl hydrazine, has long been known to have sometimes had poisonous effects upon those who have used it extensively in studying sugars by the methods devised by Emil Fischer. However, the lesions produced by hydrazine are of such a nature that this drug promises to have a field of usefulness in certain metabolic and pathologic investiga-

[1] Received for publication April 27, 1908.

[2] *Jour. of Biol. Chem.*, 1908, iv, 165.

tions. In these experiments the sulphate of hydrazine has been used, and the general effects are described by Underhill and Kleiner as follows: "The researches of Borissow, of Pohl, and of Poduschka have demonstrated the relatively great toxicity of this compound, and have defined the series of manifestations following its introduction into the body. With doses of 0.1 gram of hydrazine sulphate per kilo of body weight subcutaneously injected, vomiting is observed, which is succeeded by extreme restlessness. There is augmentation of the heart beat which later falls far below the normal, and respiratory difficulty is accompanied by general paralysis. At this stage a short period of coma usually ensues which terminates in death. The entire cycle of events is completed within a very few days. Coincident with the symptoms noted above is the appearance in the urine of variable quantities of protein and bile pigments, together with appreciable amounts of allantoin crystals. The liver appears to suffer fatty metamorphosis, and autolysis of various tissues of such experimental animals leads to the presence of significant quantities of allantoin in the digestion mixtures."

Organs from eleven dogs, two cats and four guinea pigs poisoned with hydrazine have been studied, and the anatomical changes resulting have been found to be quite constant. The poison is remarkable in that it affects only the liver, so far as the histological evidence shows, leaving all the other organs unaffected. In the liver the changes are very constant, although the degree of change has not always been found to vary directly with either the size of the dose or the duration of the poisoning, presumably because of individual variations or inconstant conditions in the dogs examined. The changes consist essentially in a fatty degeneration of the cytoplasm of the liver cells, which begins in the center of the lobules and progresses outward, until in dogs dying from three to six days after being poisoned the process has extended to involve extensively all but the peripheral cells of each lobule, and no cells are present that do not contain more or less visible fat. Only the cytoplasm of the cells is primarily affected by the poison, the nuclei remaining intact until long after the cytoplasm has become a barely distinguishable network of protoplasmic granules and threads inter-

spersed between closely packed droplets of fat; when the degeneration has reached this degree the nucleus may begin to stain faintly, enlarge, become vacuolated, and disappear, as if suffering from lack of nutrition on account of the quantity of fat surrounding it. After a time the cells in the center of the lobule lose not only their cytoplasm, but the fat that has replaced it also disappears, so that nothing remains of the liver cells in the center of the lobules but an occasional badly degenerated cell or a practically naked nucleus; the place of the cells is usually taken by compensatory dilatation of the capillaries. Often there is some fatty change in the epithelial cells of the bile ducts, but the stroma cells and the stellate cells of Kupfer are entirely unaffected. Occasionally there are present in the bile vessels homogeneous bile-stained plugs. There were never observed instances of primary destruction of the nucleus, and the features of the ordinary central necrosis of the liver produced by bacterial poisons are entirely lacking; nothing like focal necrosis has been observed, and there is little or no tendency for leucocytic accumulation in the liver.

When stained with Sudan III, the vacuolization of the liver cells is seen to be due entirely to fat, which accumulates as minute droplets, packing the cytoplasm of the most affected cells so full that it seems to be one mass of fat. The nucleus of even the most fatty cells usually appears unaltered, and retains its position in the cell, surrounded on all sides by fat. In all marked cases there are practically no liver cells that do not show more or less fat, but the demarcation between the greatly affected central portion and the less affected peripheral portion is usually quite sharp, because of a sudden transition from cells packed with fat to cells containing but a few droplets. Fat droplets are also found frequently in the epithelium of the bile ducts, but not in the cells of the stroma and blood vessels. In those cases in which the effect of the poison has been most marked, so that the parenchyma cells in the centers of the lobules have disappeared almost entirely, the fat will be found occupying the intermediate zone of the liver lobule, the cells of the vessels and stroma left in the center of the lobule being practically free from fat, and the amount of fat in the peripheral cells being small in amount in contrast with the middle zone; in such speci-

mens the histological picture is very striking, the orange Sudan III stain forming a solid band, surrounded on each side by the hematoxylin-stained nuclei of the surviving liver cells.

Hydrazine does not seem to affect organs other than the liver, although occasionally minute hemorrhages into the lung or a small amount of bloody pulmonary edema can be found. In one dog considerable hemorrhage was found in the medulla of one adrenal, and in the same dog there were numerous small interstitial hemorrhages into the pancreas with several typical foci of fat necrosis in the immediate vicinity of the organ. The lungs and kidneys are usually congested, but show no microscopic changes. In most of the dogs the brain, heart, gastro-intestinal tract, pancreas, adrenals, lymphatic system and generative organs have been examined without any changes whatever being found. Fatty changes were never found in the myocardium or kidney by special staining. Apparently hydrazine does not affect the red corpuscles, for the liver cells and the spleen and lymph glands were quite free from abnormal pigmentation. Hyaline thrombi or fibrinous deposits were never observed in even the most altered livers.

The general features of hydrazine poisoning may be shown by giving a typical protocol, as follows:

Dog A. Weight 10.83 kilos; very fat, part pug, male. January 15, 11 A. M., I injected 0.4 grm. hydrazine sulphate in 20 c.c. water, subcutaneously in left flank. The dog was a trifle indisposed the next day, but recovered by the end of 48 hours. January 18, 11 A. M., I injected 0.8 grm. hydrazine into the right flank. At this time the dog seemed perfectly well. January 19.—The dog was found dead in the cage, having apparently died about 6 A. M.

Autopsy.—Subcutaneous tissue at the site of injection is pink, but shows no evidence of infection or acute inflammation. Cultures from this point and from the heart's blood gave no growth. *Liver* was very yellow, friable in consistence, and weighed 335 grams. The centers of the lobules were red, but the rest of the lobule was strikingly yellow. The veins were full of blood. *Heart* showed no evident changes; the cavities were full of dark blood which was not coagulated. *Spleen* showed no gross changes. *Kidneys* were very much congested, with the cortical vessels particularly injected. *Stomach* showed some slight congestion of the mucosa, but no other changes were found here or in the rest of the gastro-intestinal tract. *Lungs* contained a great amount of blood but showed no definite areas of hemorrhage or consolidation.

Histological Examination.—*Liver.* The central part of each lobule when stained with hematoxylin and eosin shows a great decrease in the intensity of staining, this condition involving fully four fifths of the length of the cords

in most cases; in some lobules only a few cells at the corners remain approximately normal. This change affects almost solely the cytoplasm, the nuclei staining with nearly as great intensity near the center as at the periphery. The cytoplasmic change consists of a high degree of vacuolization which reduces the cytoplasm to a coarse network, enclosing vacuoles in size varying from droplets as large as the nucleus down to minute granules; the affected cells are somewhat increased in size. There are no cells, even at the corners of the lobules, that do not show some vacuoles. There can be found no leucocytic accumulation, no congestion, and no connective tissue proliferation. In the central area the liver cells are entirely absent, except for occasional extremely degenerated cells with but minute shreds of cytoplasm remaining; there also are many nuclei that resemble liver cell nuclei, some of which stain faintly and some appear normal. Sections cut in agar, and stained by Sudan III and hematoxylin, show each lobule to consist strikingly of three zones of about equal width. The central zone contains neither liver cells nor fat droplets that are readily recognized, but consists chiefly of surviving stroma and capillary cells, with an occasional degenerated liver cell or liver cell nucleus. The intermediate zone is extremely fatty, rather sharply defined from the central zone, and less so from the peripheral zone. Here the cytoplasm of practically every liver cell is so densely packed with fat that the individual granules cannot be distinguished; but no matter how fatty the cell the nucleus is almost always clear and well stained. Occasionally the droplets fuse and force the nucleus to one side, but more often it is central and surrounded with fat. The peripheral zone differs solely in that the fat droplets are of smaller size and less abundant, so that much of the cytoplasm can be distinguished. Some of the small bile ducts contain fat droplets in the epithelium.

Kidney shows considerable congestion, especially in the pyramids but there are no changes in the epithelium. No fat droplets are found in sections stained by Sudan III.

Lung shows many of the alveoli full of blood, which contains no fibrin and no excess of leucocytes.

Myocardium shows no changes. No fatty changes present in sections stained with Sudan III.

Adrenal shows extensive hemorrhage in the center of the medulla, but no leucocytic accumulation and no thrombosis.

No changes found in any of the other organs and tissues.

When the animals are given enough hydrazine to make them quite sick, and are then allowed to recover, the process of repair of the liver lesions seems to consist simply in a gradual decrease in the amount of fat in the cytoplasm until the cells resume their normal appearance, which requires two or three weeks. The cytoplasm of such cells has been found extensively vacuolated, but not reacting for fat, so that there is apparently some intracellular edema following removal of the fat. This recovery of greatly degenerated cells shows how specifically the poison affects the cytoplasm

without injuring seriously the nucleus, and without destroying the vitality of the cell. No evidence of regenerative proliferation of the liver cells and bile capillaries, or connective tissue overgrowth, which are so characteristic of the recovery from acute yellow atrophy, has ever been observed.

Summary.—Hydrazine seems to be a poison with an almost specific effect upon the cytoplasm of the parenchymatous cells of the liver, for when the poison is given subcutaneously this tissue alone shows evident structural alterations, although equal or greater amounts must reach other organs and tissues. It seems to have remarkably little effect upon other than hepatic cells, and does not cause any appreciable destruction of red corpuscles; slight hemorrhages are occasionally produced, but much less than by other poisons with a similar effect upon the liver. It attacks only the cytoplasm of the liver cells, never affecting the nucleus primarily, and causes a profound fatty metamorphosis of the type commonly referred to as " fatty degeneration." In this respect it resembles phosphorus, from which it differs in two important particulars. Hydrazine attacks first the cells in the center of the lobules, while phosphorus shows its first and most marked effects upon the peripheral cells; and secondly, phosphorus usually causes marked fatty changes in the myocardium, the kidneys, and indeed throughout the body, whereas the effects of hydrazine seem to be limited almost absolutely to the liver. The unknown poisons of acute yellow atrophy and eclampsia, and most of the bacterial poisons, attack first and chiefly the nuclei of the liver cells, in contrast to the strictly cytoplasmic effects of hydrazine. Phosphorus also affects the nuclei more than does hydrazine. On this account the recovery of the liver to normal after hydrazine poisoning is remarkably rapid and complete, there being no permanent anatomical alteration after recovery from a most severe non-fatal poisoning.

As a poison for use in experimental studies of hepatic metabolism, hydrazine would seem to commend itself over phosphorus on account of its more selective action upon the liver. In the maximum sublethal doses given for experimental purposes it will destroy fully as large an amount of liver tissue as will phosphorus; but there will always be left a considerable amount of liver tissue in a fair

state of preservation, and presumably functionally competent, whichever poison is used. When recovery of the experimental animal is desired the return of the liver to normal will probably be more rapid and more complete after hydrazine than after phosphorus.

To the pathologist the peculiar action of hydrazine presents many interesting problems. In the first place, why is it that hydrazine shows its effects first in the center of the lobules, while so similar a poison as phosphorus attacks first the periphery? Again, why does hydrazine act so specifically upon the liver cells, even when subcutaneously injected? And why does it limit its action so specifically to the cell cytoplasm, leaving the nucleus practically uninjured? It seems probable that the use of this drug in studies of fatty metamorphosis might throw light upon some of the obscure phases of this puzzling subject. As to the significance of the effects of hydrazine for physiology I may quote from Underhill and Kleiner, as follows: "The most striking feature of the action of hydrazine upon the animal body is the absence of abnormal relationships in the principal urinary constituents. Yet according to histological examination the liver is profoundly altered in structure and a large proportion of the cells is apparently inactive. The only inference that can be drawn from such evidence is that through the persistence of a small number of normally functionating liver cells this organ is enabled to maintain its intermediary metabolic processes in approximate equilibrium. This is in harmony with the recently published observations of Jackson and Pearce upon the production of artificial liver necrosis by injections of hematoxic sera, and as they have aptly pointed out, constitutes one of the best examples of the 'factors of safety' or protective adaptations in the animal body."

In conclusion, I take pleasure in expressing my indebtedness to Professor Underhill for the opportunity of making this study, and for much of the material that was used, as well as for his permission to publish this phase of the studies upon the action of hydrazine.

EXPLANATION OF PLATE XXVII.

FIG. 1. Showing the fat deposited about the central veins. Stained with Sudan III and hematoxylin, the fat appearing black in the photograph. Condition observed 24 to 48 hours after poisoning.

FIG. 2. Later stage than Fig. 1. About the central veins, shown black in the photograph, are only stroma and liver cell nuclei. The light areas represent corners of lobules; the fat (photographed black) occupies an intermediate zone in the liver lobule, as a band surrounding the relatively unaffected corners of the lobules.

FIG. 3. Same as Fig. 2, but higher magnification. The pale area in the center represents parts of adjacent lobules about a portal radical, which is separated by a dark colored band of fatty degeneration from the central parts of the lobules, where only a few large globules of fat remain.

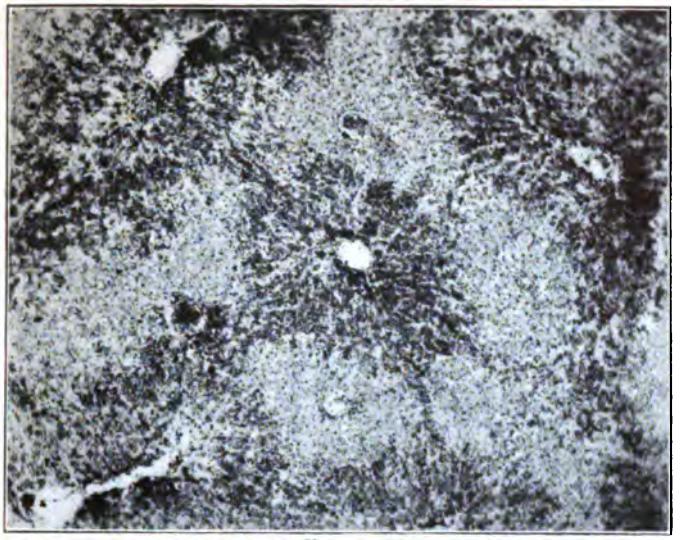


FIG. 1.

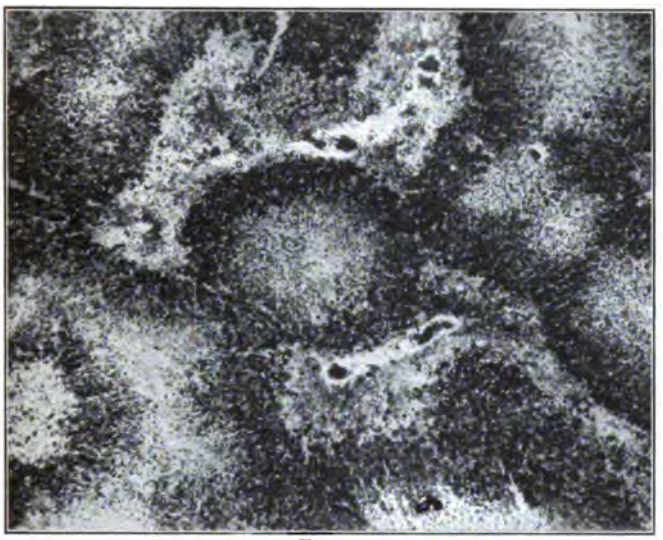


FIG. 2.

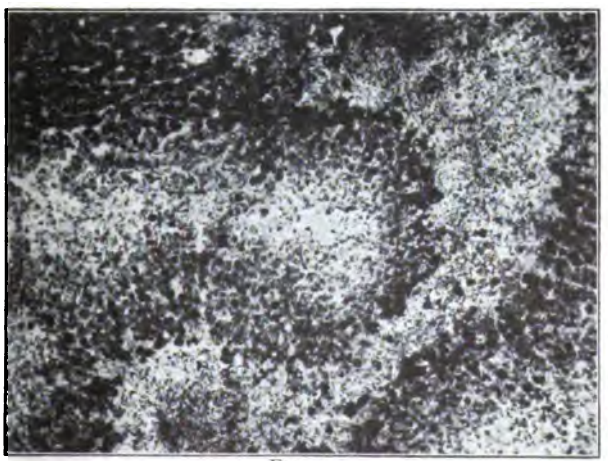


FIG. 3.

MELANOMA OF VATER'S DIVERTICULUM AND LOWER PORTION OF COMMON BILE DUCT CAUSING COMPLETE OBSTRUCTION.[1]

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(From the Pathological Laboratory of the Montreal General Hospital.)

PLATE XXVIII.

According to our present conception of pigment-producing cells melanotic tumors do not occur outside the skin, eye and central nervous system, except as metastatic growths. Pigmented tumors that arise in the internal organs are traced in almost every case to a primary growth in a situation where pigment cells normally exist. Most commonly the black moles of the cutaneous surface and the pigment cells of the choroid are responsible for these metastases. Occasionally, however, one cannot find the slightest evidence of abnormal cell activity in the normally pigmented sites, though distant parts show well-advanced melanotic growth. Hence the possibility must be borne in mind that a dislodgment of one or more pigment cells may occur at some time from a normal situation and subsequently become arrested at a distant point. In this way we may account for the occurrence of a melanoma in an internal organ where no primary tumor actually exists.

The neoplasm which is the basis of this study is definitely a melanophoroma and has occurred in the common bile duct of man. The duct was completely obstructed throughout the full extent of the growth. An obstruction of this nature and in this location is, to say the least, unique and aside from its surgical importance affords a most interesting histological study.

A careful search failed to establish the metastatic nature of the obstructing growth. The close resemblance of the cells of this tumor to epithelium renders the microscopic findings of sufficient importance to warrant a detailed description.

[1] Received for publication April 18, 1908.

The clinical history and autopsy protocol are as follows:

Patient, male, white, aged 44, was admitted to the Montreal General Hospital in the spring of 1907, suffering from acute biliary obstruction. The first symptom appeared two months prior to admission as a cutaneous irritation, especially of the skin of the feet; the patient later became jaundiced and jaundice gradually deepened and was associated with great weakness and loss of weight. There was no vomiting at any time.

Shortly after admission a biliary fistula was established and in addition to bile, small granular bodies were intermittently discharged but at no time were definite calculi noted. At the operation a slight cholecystitis but no evident cause for the biliary obstruction was found. Throughout the period of observation the stools were fatty. The patient died early in September, two months after the operation and four months after the first symptoms were noted.

Autopsy was performed fourteen hours post-mortem. The body is that of a white adult male, fairly well developed but emaciated, with marked icterus of the skin, conjunctivae and mucous membranes. Rigor mortis is present. There is slight lividity over the dependent parts. The pupils are unequal, the left being 4 mm. in diameter, the right 2 mm. There is no œdema. In the right hypochondrium is a linear scar 10 cm. in length which traverses the right rectus muscle parallel to the median line. In the line of the cicatrix is a fistulous opening communicating with the gall bladder and on pressure over the upper abdomen the fistula discharges bile. The left parotid gland is the seat of extensive suppuration. Thick purulent material is easily expressed through an existing fistula.

There are no pigmented moles on the surface of the body.

Peritoneal Cavity.—The parietal and visceral peritoneum is deeply stained with bile. The appendix is 12 cm. in length, retrocæcal and twisted upon itself in its outer third. There is no enlargement of the mesenteric or retroperitoneal lymph nodes. The height of the diaphragm is normal. In the region of the gall bladder are numerous old fibrous adhesions especialy about the fundus, formed after its attachment to the abdominal wall.

Pleural Cavities.—The right pleural cavity shows a few old fibrous adhesions over the upper lobe.

Pericardial Cavity.—There is the normal amount of fluid deeply bile tinged.

Heart.—Wt. 215 grm. The subepicardial fat is slight in amount. Pulmonary artery opened *in situ* contains post-mortem clot and fluid blood. The myocardium is of good color and consistence. The endocardium and valves are normal. The aorta and coronaries show nothing remarkable.

Lungs.—In the right upper lobe there is a small calcareous nodule the size of a pea (healed tuberculosis).

Spleen.—Wt. 150 grm. Organ shows nothing abnormal. The gastrointestinal tract and pancreas are normal. There is a firm gland 1 cm. by 0.5 cm. in the region of the cœliac axis, which on section is deeply discolored with bile, but otherwise shows no abnormality.

Liver.—Wt. 1,580 grm. The lobules are accentuated in outline because deeply bile stained; aside from this discoloration there is nothing noticeable.

Gall Bladder and Ducts.—The inner surface of the gall bladder appears

normal. The fundus is found opened and sutured to the abdominal parietes and communicates through a fistulous opening with the exterior. The cystic duct opened *in situ* is seen to be S-shaped—apparently the result of old adhesions. The wall of the cystic duct is of normal thickness and its inner surface smooth throughout. On opening the common duct it is found to be uniformly dilated to about three times its normal caliber. Its wall is somewhat thinned and the rugæ of the tunica propria present marked fenestration—presumably the result of long-continued distension. *In the lower portion of the duct there is a soft brownish black mass, 2.5 cm. in length, which completely occludes the lumen.*

Kidneys.—Wt. 260 grm. Both organs on section show a dark greenish discoloration of the cortex and medulla. The lining of the pelvis is bile stained and presents numerous small superficial hemorrhages.

The urinary bladder and genital organs are normal.

Brain.—Wt. 1,280 grm. Surface presents nothing remarkable. The pia-arachnoid shows the general deep bile discoloration. Lateral ventricles, pons, medulla, mid-brain and basal ganglia are negative. There is no evidence of abnormal pigmentation. The hypophysis, spinal cord and membranes are apparently normal.

Description of the Tumor.—The growth consists of a soft fungoid brownish black mass 2.5 cm. in length which completely fills the lower portion of the common bile duct, including part of Vater's diverticulum but without encroachment upon the duodenal passageway.

A grooved director was first passed down the lumen of the common bile duct into the gut without meeting the least resistance. Only after the duct had been laid open was the obstruction discovered. The tumor now presented itself as a soft black cylindrical mass which bulged over the edges of the opened duct. It was only with great difficulty that the tumor could be returned to its original place and the severed edges of the duct brought again into apposition.

The tumor throughout its extent was intimately attached to the duct wall and appeared to have started in the tunica propria. There was no thickening of the wall, which seemed abnormally thin. The upper and lower margins of the growth were sharply defined. The base of the growth maintained an even line 2 mm. below the surface epithelium as indicated to the naked eye by the pigment limit. The tumor was confined entirely to the common duct and ampulla. The pancreas and the duct of Wirsung were in no way involved by the growth.

At first it was thought that the tumor consisted of altered blood or inspissated bile; its true nature, however, was discovered after a more careful examination. The melanoma on closer inspection showed innumerable densely arranged flattened finger-like projections which floated free at their distal extremities, but remained firmly attached at their bases as demonstrated by immersion in water.

The tumor with its curious villous structures resembles certain forms of vegetable algæ growing under water. It is noteworthy that no part of the mass could be washed away, nor was the water discolored by the coloring matter of the tumor. On removing the mass from the water the villi immediately collapsed allowing the tumor to again assume a smooth dull black surface.

Microscopic Examination.—Longitudinal strips of the tumor throughout its whole extent were incised for microscopical study. The sections not only included the duct wall, but a part of the pancreas and the surrounding tissues. A portion of the uninvolved duct was included in the sections from the upper and lower limits of the mass in order that the outer cells and their relationship to the normal tissues might be studied. The tissues were fixed in Zenker's fluid, alcohol and formalin, and embedded in paraffin. The material, hardened in Zenker's fluid, was subsequently stained with eosin-methylene-blue for the routine study. Mallory's connective tissue, phosphotungstic-acid-hæmatoxylin and fibroglia stains were employed for the differentiation of special tissues. The material preserved in alcohol was used for the determination of iron-containing pigment; the test, however, was negative.

The growth, in general, under low magnification presents an alveolar structure which is irregularly and loosely formed. In some parts the cells are densely packed, while in others they are loosely arranged without any intercellular supporting tissue.

Sections treated with Mallory's special stains for connective tissue and fibroglia fail to show either of them. As a whole the tumor is without intercellular supporting tissue; an exception to this statement are some alveoli in the periphery of which the lining cells rest upon a delicate basement membrane. This membrane where present consists of fibers which run lengthwise to the glands and contain widely separated flattened cells from which the fibers are apparently derived. In addition to the cell groups which possess these supporting fibrils, there are those which apparently are held together only through cellular cohesion.

I believe that these basement supporting fibers and their cells are the same as those described by Mallory (1) as forming the true basement membrane of adeno-fibromata of the breast. As he points out, these fibrils always accompany the epithelium of the newly formed glands (adenomata) and seem to form as much a part of the tumor as the epithelium.

It is of interest to note that the villous masses comprising the tumor are covered by a thin though well-defined connective tissue envelope, in whose fibrils are elongated cells arranged end-to-end

in an unbroken chain. There is no pigment either intra- or extracellular in this supporting tissue, and in no way does it resemble the melanoma.

The growth apparently started in the gland follicles of the tunica propria where the pigment cells are more numerous within and around the lining epithelium than in any other part of the involved duct wall. Microscopic examination shows that the pigment cells do not extend below the level of the gland follicles, and are so intimately associated with the epithelium of the follicles that it is impossible to distinguish them. This is so striking in some sections that one is inclined to regard the growth as epithelial in origin. Either the normal gland epithelium has become phagocytic to melanin granules of neighboring mesoblastic cells or the epithelium here represents a malignant metamorphosis. Certainly in no portion of the duct throughout the entire basal length of the tumor can one find pigment-producing cells that have involved the vessels beyond the tunica propria.

Many of the glands are composed of single layers of evenly arranged columnar cells that show all grades of pigmentation. Occasionally follicles are lined with two distinct types of cells; the one columnar, mononuclear and non-pigmented; the other bizarre in outline with two or more nuclei and protoplasm completely filled with pigment granules. These two types of cells alternate to form the basal layer of the mucous glands. Possibly these chromatophors should be regarded as foreign cells which have invaded the gland epithelium, since they differ in size, shape and position from the normal columnar cells of the follicles.

These pigmented cells with their long protoplasmic processes frequently alternate with the non-pigmented epithelium, and in places the cell body may be seen pushing its way through the basement membrane of the gland or projecting into the lumen. The processes filled with pigment stream out like tentacles between the normal columnar epithelium. Invariably the protoplasmic prolongations are directed toward the gland lumen; only rarely, indeed, do they point in the opposite direction, though the cell body proper is often beyond the basement membrane or even in the periglandular space.

As a rule the outline of each pigment cell is sharply defined by the brownish black granules, which completely fill every part. The whole is a striking picture of abnormal epithelial activity and suggests that the gland follicle is probably the true generic center of the melanophoroma.

The cells which are free in the lumen of the gland are decidedly angular in outline, especially where they are crowded together. This appearance is even more noticeable in the cells lying free in the alveoli of the melanoma (see Plate XXVIII, Fig. 3). Commonly they are hexagonal, though the chromatophors not associated with an alveolus are round or ovoid. In neither situation, however, are they provided with the protoplasmic extensions which are so characteristic of the pigment cells found in the normal gland follicles. In general the size of the ovoid and hexagonal cells varies greatly, the larger ones measuring 40 to 60 microns and the smaller 4 to 8 microns in diameter.

The new growth is almost entirely composed of pigmented cells; only occasionally are the cells non-pigmented and here apparently they do not belong to the normal tissues. The pigment is most marked in the outer extremities of the villi where the alveolar structure is most pronounced.

The cells have a large distinctly lobulated vesicular nucleus with one or more nucleoli. The nucleolus is always sharply defined and may be very large, sometimes the size of the nucleus of a small lymphocyte. There is often only a narrow rim of nuclear material surrounding an enormous nucleolus. The great variation in size and density of the nucleoli is another prominent feature of the tumor. It is not uncommon to find cells with two or more nuclei held together by narrow bands of basophilic material, each nucleus provided with its nucleolus and normal amount of chromatin.

Mytotic figures are only occasionally found and then in cells sparsely provided with pigment. In certain amœboid cells there may be seen a nucleus in the protoplasmic extension, while a second exists in the cell proper.

Pigment production is apparently in direct proportion to cell activity; the large active cells with deeply staining nuclei containing an abundance of pigment, the small cells with pale nuclei containing

but few granules. The pigment generally occurs in the form of globules arranged in the cell protoplasm equidistant from one another. These globules vary greatly in size, from mere points to that of the ordinary eosinophilic granule. Their color ranges in tint from a light mahogany to a dark blackish brown.

In many cells the pigment granules have coalesced to form one large globular mass which is either centrally or excentrically situated. About such a mass there are apparently as many pigment granules as in the cells of the same size which show no fusion of the pigment. Often the coalesced pigment forms a dense brownish black mass which occupies the inner two thirds of the cell, whilst the periphery is formed by a narrow band filled with light brown granules. Large vacuoles are seen in some cells and undoubtedly represent spaces where the coalesced pigment has dropped out. Many cells are so densely crowded with the granules that the nucleus is not discernible even on change of focus.

The nuclei often contain masses of chromatin-like material which strongly resembles melanin. Whether an excess of nuclear pigment is thrown out and constitutes subsequently the pigment of the protoplasm cannot with certainty be determined.

The pigment differs from that of the melano-sarcoma in that it occurs in the form of evenly arranged intracellular globules instead of irregular scattered masses. The distribution is so regular that it does not seem to be a by-product, but rather an integral part of the cell.

Some chromatophors are phagocytic to others and, in such instances, are easily distinguished from the common endothelial scavenger of blood pigment. The inclusion often fills the greater part of the phagocytizing cell, displacing its nucleus to the extreme periphery. Sometimes there is a broad clear zone about the engulfed cell, outside of which is the protoplasm of the phagocyte.

Occasionally a small vessel in the submucosa of the common bile duct and in close proximity to the tumor contains pigment cells. These cells have the same general morphology and are filled with dark brown granules similar to those of the cells above described. They are not free in the lumen, but appear from their close relation to the intima to be part of the lining endothelium, except that they

are deeply pigmented and bulge into the lumen far beyond the normal cell line. With the exception of these vessels near the tumor the melanotic cells are strictly confined to the main mass. Although some vessels contained chromatophors, contrary to what might be expected, no metastases were found.

A few eosinophile, lymphoid and plasma cells are scattered through the basal portion of the tumor. Below the region of the involved part of the duct there is a well-marked peri-glandular inflammation of lymphoid and plasma cells, but no pigmented cells of any description. Here the cylindrical epithelium of the glands is normal in appearance, though it is evident from the leucocytic infiltration that the follicles are the seat of a chronic inflammation. As far as can be determined all the gland follicles in the tunica propria above and below the growth show a low grade of chronic inflammation, though the other tissues are normal. This fact, in view of the inadequate explanation of the occurrence of this melanoma, strongly inclines one toward Ribbert's (2) theory that there may be some connection between the tumor and this inflammation.

On microscopic examination the pancreas, liver and other organs of the body show nothing remarkable.

Discussion.—The melanophoroma here described is of considerable histopathological as well as surgical interest. We not only have a pigmented tumor unique in its situation, and apparently primary in the common bile duct, but one whose cellular structure resembles epithelium in many ways.

Though secondary growths from melano-sarcoma are common in the liver there is, to my knowledge, no reported case of such a growth occurring in the common bile duct. Statistics show that the gall bladder and ducts are far more frequently the sites of primary non-pigmented sarcomata. In view of this fact the presence of melanin in the tissues where normally it does not exist, as in this case, might impose an insuperable objection to the belief that this is a primary growth. The tumor under consideration, it would seem, originated in the normal cells of the wall of the common bile duct, for the most careful search of the cutaneous surface and central nervous system failed to show anything that would

indicate its metastatic nature. It is to be borne in mind, however, that there is the possibility of a dislodgement at some time of one or more pigment-bearing cells which subsequently have been arrested at the site of growth. In support of this one can advance the recognized metastasis of thyroid cells in remote parts of the body in cases where there is no primary growth in the gland itself.

There are many features of the tumor which suggest an epithelial origin; on the other hand, there are points in favor of its being an alveolar melano-sarcoma. In considering an epithelial histogenesis one is struck with (1) the unusual situation of the growth in a tissue where melanoma have never before been noted, (2) the epithelioid character of the cells and their tendency to mimic in arrangement glandular structures, (3) the intimate association of the cells with those of the normal gland follicles, and (4) the unusual regularity in size, color and distribution of the pigment in each cell, compared with that of the ordinary melano-sarcoma. Of course it is difficult to conceive that a primary melanoma, no matter whether mesoblastic or epiblastic, could arise from cells in this location.

Undoubtedly the strongest point in favor of sarcoma is the presence of melanin, though even here the pigment is atypical in its arrangement and distribution. Ordinarily in melano-sarcoma there are areas that contain no pigment, while others are sparsely supplied and in still other areas pigment is in considerable amount.

If we disregard the pigment for the time, the nature of the growth is only to be determined by its structure and cell morphology. After all the morphological character of the cell is of little value as an aid toward classifying tumors, especially where there is no definite arrangement. The inter- and intracellular fibrils produced by cells certainly offer a more reliable means. In this melanoma of the common bile duct the cells in many parts are similar in morphology to glandular epithelium. They show, moreover, a decided tendency to form alveoli; this fact, considered with the epithelial character of the cells, would make it impossible to class the tumor among the sarcomata were it not for the presence of melanin.

The question naturally arises, can we have a melanophoroma

either meso- or epiblastic in nature which is primary in a situation where normally there are no pigment cells? It is conceivable that epithelial as well as mesothelial cells normally not pigmented may produce pigment under certain conditions.

Hertwig (3) suggests that the pigment of melano-sarcoma is formed from chromatin extruded from the nucleus in an effort of the cell to reduce its nuclear mass, and thus to reorganize itself. In a later paper (4) he describes the transformation of chromidia into pigment. Rössle (5) claims to have traced in the cells of melano-sarcoma the wandering of nuclear substance from nuclei over-rich in this material, and its transformation into pigment in the protoplasm. He suggests that the pigment formation of senile atrophy is produced in the same way as the pigment of actinosphærium and shows, as far as microchemical methods go, the pigments of actinosphærium, melano-sarcoma and brown atrophy to be apparently identical and iron free. Meirowitz-Grandenz (6) in their study of pigment formation in the skin after exposure to light were able to trace both in the nucleus and in the protoplasm pigment from nucleolar substance.

I have observed the formation of autochthonous pigment in corneal epithelium where the cells under toxic irritation become filled with pigment granules. Whether or not this pigment is altered and extruded nucleolar material I am not prepared to say. In consideration of the above facts, I am inclined to believe that the tumor here reported has its origin in cells of the common bile duct.

There is also much evidence in favor of an epithelial histogenesis, especially when we consider that the cells do not produce fibrils or intracellular fibers. Also the more recent work on melanin-producing cells tends to strengthen the conception of their epithelial origin. The pigment may be the result of some peculiar action of certain products of liver metabolism on the mucous gland cells in the common duct, and, as Hertwig suggests, represents the by-product of over-active cells in their attempt at reorganization.

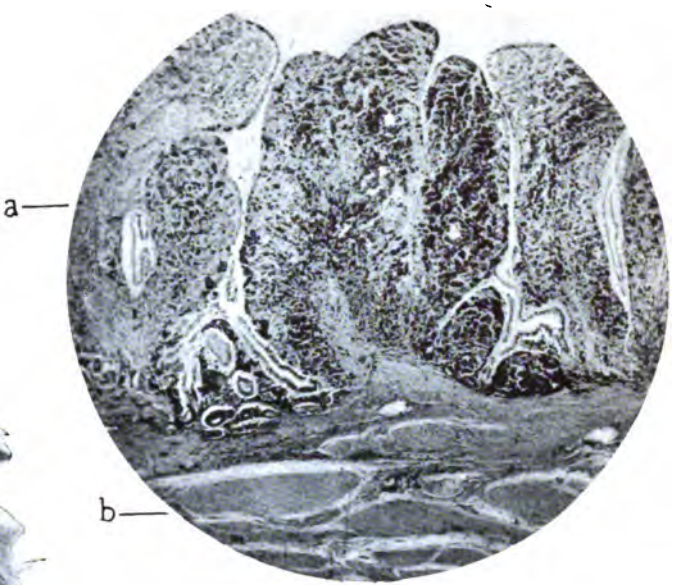

FIG. 2.

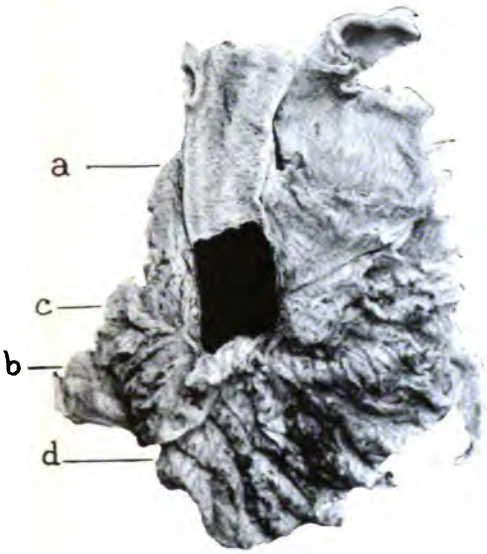

FIG. 1.

FIG. 3.

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Explanation of Plate XXVIII.

Fig. 1. Gross appearance of the tumor *in situ.* (*a*) Upper part of the common bile duct. (*b*) Ampulla of Vater. (*c*) Obstructing melanotic growth. (*d*) Duodenum.

Fig. 2. Section through the common bile duct including the melanoma under low magnification. (*a*) Pigmented villous projections which in parts show an alveolar construction. (*b*) Muscularis of the common duct.

Fig. 3. Represents a camera lucida drawing, Leitz, ocular 3, immersion lens 1/16. Note size, shape and arrangement of the pigmented cells.

A BIOLOGICAL STUDY OF THE CEREBRO-SPINAL FLUID IN ANTERIOR POLIOMYELITIS.[1]

By MARTHA WOLLSTEIN, M.D.

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During the spring and summer of 1907 several hundred cases of acute anterior poliomyelitis occurred in New York City and vicinity. Towards the end of the summer and in the early autumn a study was made at the Rockefeller Institute of certain of these cases. My part of the study consisted of an effort to find evidence of a micro-parasitic origin of the disease by the employment of the biological reaction of complement deviation according to the methods of Bordet (1) and Wassermann and Bruck (2). This reaction or test has been applied now to the study of a number of infectious diseases, among which are tuberculosis (2), typhoid (3), gonorrhœa (4), and for the identification of particular antigens and antibodies as in syphilis (5) and pertussis (6), with a certain measure of success. The deeper the study is pursued the less convincing has it become that the reaction depends upon specific antibodies in the real sense, and the more probable has it become from the work of Landsteiner, Müller and Pötzl (7) and of Porges and Meier (8) that the lipoids of the blood and organs take an essential part in the reaction. Notwithstanding this, however, it may still display a high degree of specificity.

When this study was begun our knowledge of the conditions of the test was less complete than it is at present. But the methods employed are those still followed, and hence the results of the study are in no way affected by the change of view in respect to the manner in which the complement deviation is produced. The idea from which I proceeded is this: If the disease is due to an hitherto undiscovered microörganism which cannot be detected microscopically, it may still be possible to find evidence of its presence by demonstrating its specific antigen. Since the disease is localized in

[1] Received for publication April 2, 1908.

the spinal cord and brain it was considered not improbable that the antigen might find its way into the cerebro-spinal fluid. By selecting therefore early cases of the disease and performing lumbar puncture this antigenous fluid could be obtained. Having assumed an infectious antigen-producing origin of the disease, the next assumption naturally was that during recovery antibodies to the antigen were formed which were to be discovered in the blood serum and possibly in the spinal fluid itself.

The hypothetical basis for experimental work was thus easily secured and could readily be subjected to a test. For this purpose a number of cases of acute anterior poliomyelitis were available. They were obtained from the Babies' Hospital, the Foundling Hospital, the Hospital for the Ruptured and Crippled, and from the private practice of Dr. L. Emmett Holt. To Dr. Holt and to the house-staff of the hospitals I am greatly indebted for this material. Towards the close of the epidemic an autopsy was secured on a child of two and a half years of age, who had eight weeks previously been admitted to the Babies' Hospital for anterior poliomyelitis and seven weeks later developed symptoms of tuberculous meningitis, to which he succumbed. The microscopic examination showed a localized, characteristic and nearly-healed poliomyelitic lesion in the lumbar part of the cord. Several different tissues from this case were employed in carrying out the tests. The case was not an ideal one for the purpose because of the association of tuberculosis with the other condition, and had the results been positive, they could not have been accepted without confirmation with tissues from pure cases of the disease. In spite of the general negative character of the experiments performed, it is considered desirable to record them on account of their bearing on the etiology of this mysterious disease.

Cerebro-spinal fluid was obtained by lumbar puncture from cases in the second day to the eighth week of anterior poliomyelitis, as follows:

2d day	1 case	11th day	1 case
4th day	2 cases	12th day	1 case
5th day	1 case	3d week	6 cases
6th day	1 case	4th week	1 case
7th day	2 cases	5th week	1 case
10th day	2 cases	6th and 8th week (same case)	1 case

The patients ranged from five months to eight years in age, nine being over two years and four under one year old.

In every instance the fluid was clear and colorless, unless mixed with blood. In about half the cases a small, conical coagulum appeared at the top of the fluid after twenty-four hours' incubation. This did not, in a single specimen, extend throughout the entire length of the liquid. Smears were prepared at once, after centrifugalizing five to thirty minutes, and after twenty-four hours' incubation in the thermostat. The smears were stained with methylene blue and by Gram's method, but cellular elements were entirely absent in fourteen cases. In six cases one to three mononuclear leucocytes were found, but large numbers were never present, and the fluid gave evidence that it was not of inflammatory origin.

Cultures were made on rabbits' blood agar, human ascitic fluid agar, glucose agar, plain agar and in bouillon. Plates were incubated anaërobically as well as aërobically. In fifteen cases the fluid proved to be sterile by both methods. In five a growth occurred. One was identified as a white staphylococcus. One was a short, Gram positive bacillus, and three proved to be a large, Gram positive coccus appearing in pairs and tetrads within large groups. The organism grew abundantly on all ordinary culture media, and is apparently similar to the coccus described by Geirsvold (9) as present in twelve of the cases studied during the Norwegian epidemic of poliomyelitis during the years 1903–06, and again in three of the cases reported by Harbitz and Scheel (10) during the same epidemic. The fluids in my series which contained the diplococci were removed from cases on the fifth day, sixth day and in the fifth week after the onset. In the first two it was present in one specimen of fluid only, subsequent punctures withdrawing sterile fluid. In the third case the coccus was cultivated from a second specimen obtained three days after the first, or on the eighth day of the disease; but not from two later fluids from the same patient. It was looked upon as a contamination.

Geirsvold (9) is of the opinion that the coccus is practically identical with the one described by Jaeger (11) and by Heubner (12), differing materially from the meningococcus of Weichselbaum and from the pneumococcus. He was able to grow it in pure culture

from the blood (number of times not stated) in cases of acute poliomyelitis anterior. Nevertheless he takes a conservative view of its etiological importance, since so little is known of the bacteriological conditions in the central nervous system under normal conditions and in diseases other than poliomyelitis.

Bülow-Hansen and Harbitz (13) found the same organism in one case, their animal experiments proving the cocci to be non-virulent, as were those isolated from the cases of Harbitz and Scheel (10). Geirsvold (9) had produced atrophy, paralysis, emaciation and death in white mice by inoculating them with cultures of the diplococcus isolated by him.

Schultze (14) looked upon the diplococci he saw in smears from the cerebro-spinal fluid in two cases of anterior poliomyelitis as meningococci, although he could not make them grow. Engel (15) obtained a growth of Staphylococcus albus from the clear fluid of one case. Concetti (16) studied ten cases and found that the fluid was sterile in six of them, while meningococci were present in one and pneumococci in two.

Pasteur, Foulerton and Maccormac (17) studied a case of acute poliomyelitis in a boy thirteen and a half years old. Almost the entire motor system was involved. Four lumbar punctures were made. The fluid, clear at first, developed a grayish sediment of lymphocytes. In the first two fluids a Gram positive diplococcus was found, which appeared occasionally in tetrads and once in short chains, but never intracellularly. The coccus could not be made to grow on any culture medium incubated both aërobically and anaërobically. Intra-cranial inoculation in rabbits was followed in one instance by paralysis on the fiftieth day, and the diplococci were found in the spinal fluid when the animal died on the fifty-third day. A second rabbit inoculated from this one developed the same late paralysis, and the cocci were present in its spinal fluid on the fiftieth day. A third rabbit inoculated with the spinal fluid of the second animal remained well. The authors believe that their case is a sporadic example of the disease which Geirsvold studied epidemically. It is apparent, however, that there is a complete absence of uniformity in the bacteriological examinations of the spinal fluid in cases of anterior poliomyelitis thus far recorded.

Only one of my cases came to autopsy. It was that of a boy two and a half years old, who died of tuberculous meningitis two months after the onset of his poliomyelitis. At the post-mortem examination an area of softening was found in the lumbar cord, extending over three centimeters in length and affecting only the right anterior horn. The case was complicated with a general miliary tuberculosis involving the cerebro-spinal meninges, pleura, lungs, spleen, liver, lymph nodes and intestines. Microscopic examination of the lesion showed it to be in the sub-acute stage with fragmentation and disappearance of the motor nerve cells, none being left intact in the right anterior horn. The blood vessels were congested and there was marked emigration of leucocytes from them. The glia cells had proliferated. In the membranes were small collections of mononuclear cells, apparently early tubercles in which a few epithelioid cells were also apparent. The lesion in the anterior horn was limited there, and did not extend to the posterior horn nor across the median line. Beyond an irregular emigration of leucocytes just outside the lesion the white matter was not involved.

The remainder of the cord was ground up, dried and extracted, as were all the organs, the sciatic nerves and the muscles of the leg,[2] according to the method of Marie and Levaditi (18).

Blood was obtained from the median basilic vein in seven cases, five to ten cubic centimeters being drawn off. Two normal children were bled as controls.

Cultures made from the blood in two acute and early cases were negative, and smears from the blood showed no evidence of abnormal cellular elements nor of any parasites.

Complement binding experiments were made with cerebro-spinal fluids from seven cases, drawn on the second, fourth, sixth and tenth days and in the third, sixth and eighth weeks, the fluids having been inactivated and proven not to be anti-hæmolytic in dilutions of 0.2 cubic centimeter.

The fluid from the most recent case (second day) was tested against the blood serum of cases suffering from the disease for

[2] These muscles were used because Dr. Meltzer had suggested that the paralyzed muscles might be the earliest seat of the lesion.

five days, three weeks and eight weeks respectively. Dilutions of 0.2 to 0.005 cubic centimeter were employed (Table I). There

TABLE I.

Immune Serum.	G. P. Complement.	Antigen.	Hen's Corpuscles.	Anti-Hen Serum.	Result.
1. 5th day case 0.1	0.05	*1. C. Sp. Fl. 2d day* 0.2	0.05	0.005	++
0.05	"	"	"	"	++
0.01	"	"	"	"	++
0.005	"	"	"	"	++
0.001	"	"	"	"	++
2. 3 weeks 0.1	"	*2. C. Sp. Fl. 2d day* 0.2	"	"	++
0.05	"	"	"	"	++
0.01	"	"	"	"	++
3. 8 weeks 0.1	"	*3. C. Sp. Fl. 2d day* 0.2	"	"	++
0.05	"	"	"	"	++
0.01	"	"	"	"	++

was complete hæmolysis in all, no binding of complement being apparent. Results with the blood serum of normal children were identical.

This early cerebro-spinal fluid (second day after the onset) was also tested with fluid from a late case (eighth week), but again no inhibition of hæmolysis occurred.

In four cases the serum was tested against the cerebro-spinal fluid from the same case, as well as against that from cases in more recent and more advanced stages of the disease. The results were uniformly negative, no binding of complement taking place (Table II).

TABLE II.

Immune Serum	G. P. Complement.	Antigen.	Hen's Corpuscles.	Anti Hen Serum.	Result.
1. 10th day case 0.1	0.05	*1. C. Sp. Fl. same case* 0.2	0.05	0.005	++
0.05	"	"	"	"	++
0.02	"	"	"	"	++
0.01	"	"	"	"	++
0.005	"	"	"	"	++
0.001	"	"	"	"	++
2. 10th day case 0.1	"	*2. C. Sp. Fl. 2d week* 0.2	"	"	++
0.05	"	"	"	"	++
0.01	"	"	"	"	++
0.005	"	"	"	"	++
3. 10th day case 0.1	"	*3. C. Sp. Fl. 3d week* 0.2	"	"	++
0.05	"	"	"	"	++
0.01	"	"	"	"	++
0.005	"	"	"	"	++

Extracts made from the medulla, spinal cord, sciatic nerves, liver and tibial muscles were tried with the serum from cases of poliomyelitis in the eighth week, third week, and on the tenth day after the onset, as well as with serum from a normal child of the same age. Complete hæmolysis was the result in every instance (Table III). In combination with the spinal fluid from a case in the

TABLE III.

Anti-Serum.		G. P. Complement.	Antigen.		Hen's Corpuscles.	Anti-Hen Serum.	Result.
1. 6th week	0.1	0.05	*1. Sp. Cord Extract*	0.2	0.05	0.005	++
	0.05	"		"	"	"	++
	0.01	"		"	"	"	++
2. 3d week	0.1	"	*2. Sp. Cord Extract*	0.2	"	"	++
	0.05	"		"	"	"	++
	0.01	"		"	"	"	++
3. 10th day	0.1	"	*3. Sp. Cord Extract*	0.2	"	"	++
	0.05	"		"	"	"	++
	0.01	"		"	"	"	++
4. Normal case	0.1	"	*4. Sp. Cord Extract*	0.2	"	"	++
	0.05	"		"	"	"	++
	0.01	"		"	"	"	++

eighth week, the results were the same. Varying the amount of antigen from 0.2 to 0.001 cubic centimeter made no change in the resulting hæmolysis. Tests with extracts from the organs of a normal fœtus, similarly prepared, gave identical results.

TABLE IV.

Anti-Serum.	G. P. Complement.	Antigen.	Hen's Corpuscles.	Anti-Hen Serum.	Result.
0	0.05	0.2	0.05	0.005	++
0.2	0	0.2	"	0.005	—
0.1	0.05	0	"	0.005	++
0	0	0	"	0.005	—
0	0.05	0	"	0.005	++
0	0	0.4	"	0	—
0.2	0	0	"	0	—
0	0.05	0	"	0	—
0	0	0	"	0	—

All the controls listed in Table IV were made for every series of tests.

It is to be regretted that no autopsy was obtainable in an early and uncomplicated case.

The results obtained show that no two interacting substances, presumably antigen and antibody, capable of uniting and anchoring complement were demonstrable in the blood serum, cerebro-spinal fluids and organ extracts studied. Therefore the diagnosis of poliomyelitis by means of a serum reaction is apparently not possible, and no light could be thrown on the etiology of the disease by this reaction.

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THE PULSE PRESSURE AS AN INDEX OF THE SYSTOLIC OUTPUT.[1]

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Plates XXIX and XXX.

In a communication made some time ago, one of us (Dawson)[2] called attention to the propriety of considering the pulse pressure (that is the difference between the systolic and diastolic pressure) as an index of the systolic output. In this connection it was stated that ". . . experiments on animals, although not yet completed, have been carried far enough to show" that "the pulse pressure varies with the systolic output. In this series of experiments the pulse pressure as determined with the Hürthle manometer was compared with the systolic output as determined with the cardiac plethysmograph of Henderson, and it was found that on stimulation of the peripheral end of the vagus, in asphyxia, hemorrhage, infusion and so forth the pulse pressure and the systolic output vary together." These experiments have now been completed and the results are presented in the present communication.

METHOD.

Anesthesia.—Each dog received from 0.6 to 1.2 grm. of morphia hypodermically, and an hour later was anesthetized with ether, administered at first with a cone, but later through a tracheal cannula.

Recording.—Simultaneous tracings were obtained as follows:

1. The mean blood pressure in the femoral artery by means of a mercury manometer.
2. The systolic output by means of Henderson's cardiac plethysmograph.

[1] Received for publication March 20, 1908.
[2] *British Med. Jour.*, 1900, ii, 996.

3. The blood pressures in the carotid and femoral arteries by means of two[3] Hürthle manometers.

The recording surface was a Hürthle kymographion and the general character of the tracings obtained is shown in Fig. 3 (Plate XXIX) and Fig. 4 (Plate XXX).

RESULTS.

The results of the experiments are so concordant that it has been deemed admissible to present only the protocols of two typical

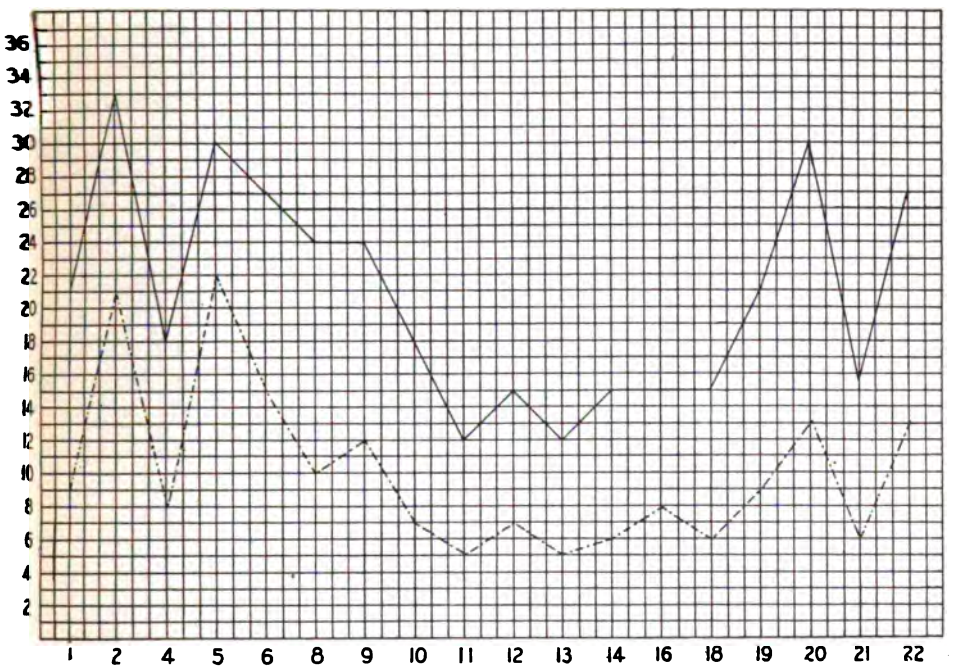


FIG. 1.

experiments. These are given in the form of two tables, two charts and two tracings.

[3] By using two arteries and two manometers we doubled the chance of obtaining an accurate record of the pulse pressure. In Fig. 3 (Plate XXIX) and Fig. 4 (Plate XXX), for example, the record obtained from the carotid was not to be relied upon, for after the completion of the experiment it was found on careful examination of the record, that with high pressures the lever pressed too tightly against the paper, while with lower pressures the lower part of the curve was spoiled by an obstruction to the movement of the lever.

Tables.—The numbers in Column I correspond to those which appear running horizontally at the bottoms of Figs. 1 and 2. The figures in Column II have only a relative value. The figures in Column III denote in cubic centimeters the change in the volume of both ventricles. Since most persons conceive of changes occur-

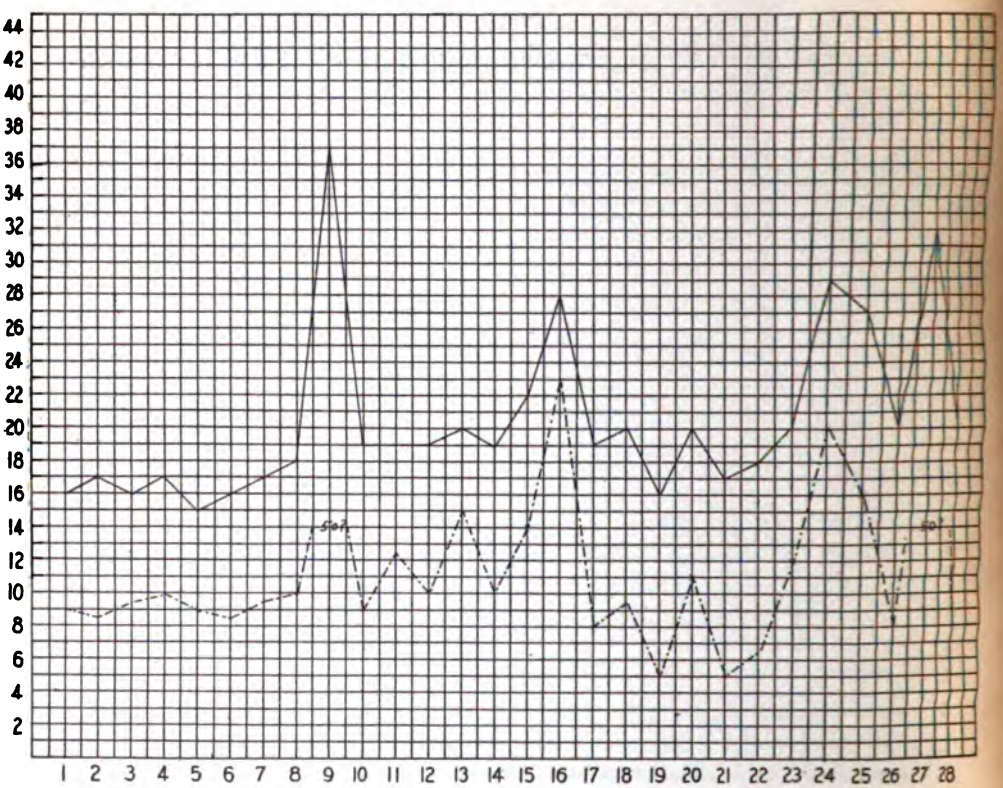


FIG. 2.

ring in the circulation in terms of variations in the mean blood pressure, Column IV has been added to complete the picture. The results given in the tables are also shown in Figs. 1 and 2, which correspond to Tables I and II respectively.

Other explanatory data are the following: asphyxia was produced by stopping the artificial respiration; stimulations were made with the faradic current; the stimulations of the annulus Vieussen-

tis occurred in the course of an ineffectual attempt to reach the accelerators. The carbonate solution had the composition: Sodium carbonate, 7.1 per cent. and sodium bicarbonate, 4.6 per cent.

TABLE I.

Experiment I.

I.	II.	III.	IV.	
Numbers Correspond to Those in the Figure.	Pulse Pressure (Length of Stroke of Hürtle Lever) from Carotid.	Systolic Output (Change in Vol. of Heart in c.c.).	Mean Blood Pressure (Femoral) in mm. Hg.	
1	7	9	158	After section of both vagi.
2	11	21	60	During stimulation of peripheral end of vagus.
4	6	8	110	After recovery.
5	10	22	54	During stimulation of peripheral end of vagus.
6	9	15	66	During escape from vagus inhibition.
8	8	10	98	Normal.
9	8	12	148	During asphyxia.
10	6	7	100	During stimulation of the central end of the vagus.
11	4	5	54	The same.
12	5	7	78	The same.
13	4	5	136	During stimulation of the annulus Vieussentis.
14	5	6	84	After recovery.
16	5	8	80	After intra-arterial infusion of strong sodium carbonate and bicarbonate.
18	5	6	64	During apnea following the infusion.
19	7	9	90	After a second infusion.
20	10	13	78	After a third infusion.
21	5	6	32	After bleeding.
22	9	13	30	After a fourth infusion.

In this experiment (Experiment I), the record obtained by the Hürthle manometer connected with the carotid artery was the more satisfactory and consequently the values relating to this artery only have been presented. Strong carbonate (see No. 18) usually causes very violent respirations followed by a respiratory pause which usually continues until death unless the animal is kept alive for some time with artificial respiration so that the effect of the carbonate has had time to disappear.

On perusal of the tables, it is seen that the pulse pressure (Column II) and the systolic output (Column III) vary together. This is shown even more clearly in the figures, where the lines which represent the quantitative variations in these two phenomena are seen to be almost exactly parallel.

TABLE II.

Experiment II.

I.	II.	III.	IV.	
Numbers Correspond to Those in the Figure.	Pulse Pressure (Length of Stroke of Hürthle Lever) from Femoral.	Systolic Output (Change in Vol. of Heart in c.c.).	Mean Blood Pressure (Femoral) in mm. Hg.	
1	16	9	97	Normal.
2	17	8	148	After section of vagus.
3	16	9	139	
4	17	10	228	Stimulation of central end of vagus.
5	15	9	126	
6	16	8	130	
7	17	9	228	Stimulation of central end of vagus.
8	18	10	144	
9	36	50?	52	Stimulation of peripheral end of vagus.
10	19	9	134	
11	19	13	164	Stimulation of the anterior crural.
12	19	10	140	
13	20	17	166	During stimulation of annulus Vieussentis.
14	19	10	130	
15	22	14	220+	Asphyxia.
16	28	28	190+	Asphyxia.
17	18	8	148	
18	19	9	168	
19	16	5	100	Bleeding.
20	20	11	182	Transfusion (intravenous) of 0.7% sodium chloride solution.
21	17	5	124	Bleeding.
22	18	6	84	
23	21	12	64	Intra-arterial infusion of strong sodium carbonate and bicarbonate.
24	29	21	82	Same.
25	27	16	106	Same.
26	20	8	58	
27	32	50?	54	Intra-arterial infusion of strong sodium carbonate and bicarbonate.
28	20	9	58	

In this experiment (Experiment II), the record obtained by the Hürthle manometer connected with the femoral artery was the more satisfactory and consequently the values relating to this artery only have been given. The very great excursions of the plethysmographic lever (see Nos. 9, 16 and 27) was probably due to the inertia of this part of the apparatus. The curve of the mercury manometer (Nos. 15 and 16) ran into the plethysmographic curve so that the height of the mean blood pressure could not be measured.

CONCLUSIONS.

On the basis of these facts we feel justified in making the following assertion:

PLATE XXIX.

Carotid (Hürtle).

Zero.

Cardiac plethysmograph.

Mean blood-pressure (femoral).

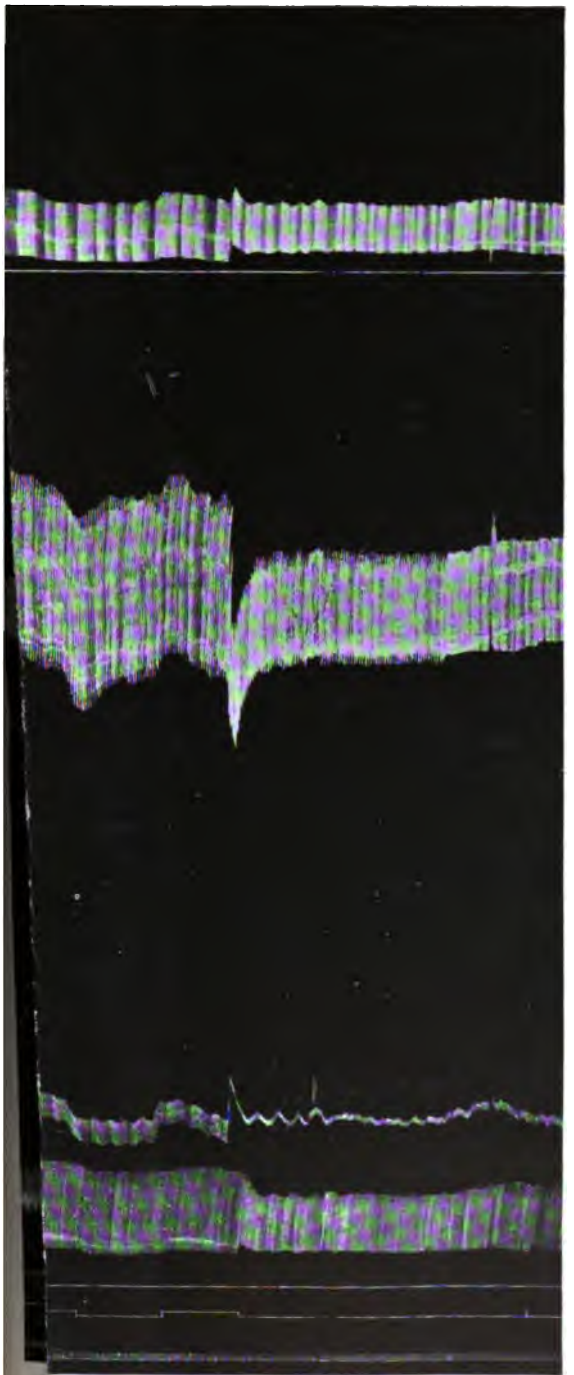
Femoral (Hürtle).

Zero for Hg. manom. and femoral (Hürtle).

Signal (stimulation).

Seconds.

PLATE XXX.



Carotid (Hürtle).

Zero.

Cardiac plethysmograph.

Mean blood-pressure (femoral).

Femoral (Hürtle).

Zero for Hg. manom. and femoral (Hürtle).

Under normal conditions and during various procedures (namely, stimulation of the vagus centrally and peripherally, of the saphenus nerve centrally, and of the annulus Vieussentis, intravenous transfusion of 0.7 per cent. sodium chloride solution, intra-arterial transfusion of strong carbonate, bleeding and asphyxia) the pulse pressure is a reliable index of the systolic output.

Explanation of Figures.

Figs. 1 and 2 are diagrammatic presentations of Tables I and II respectively. The solid line represents pulse pressure; the broken line, systolic output. Fig. 3 (Plate XXIX) and Fig. 4 (Plate XXX), are parts of the tracing obtained in Experiment II.

Plate XXIX.

1 and 2. Stimulation of central end of vagus.
3 and 4. Stimulation of peripheral end of vagus.

Plate XXX.

1 and 3. Intra-arterial transfusion of strong carbonate.
2. Bleeding.
4, 5 and 6. Stimulation of central end of vagus.

STUDIES IN RESUSCITATION: IV. THE RETURN OF FUNCTION IN THE CENTRAL NERVOUS SYSTEM AFTER TEMPORARY CEREBRAL ANÆMIA.[1]

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Contents.

I. INTRODUCTION.

The experiments on the resuscitation of the central nervous system were begun more than three years ago. The primary object was to test the possible length of time elapsing after death, after which the return of all the nervous processes could be hoped for, since we had good reason for believing that the central nervous system would prove a weak link in the vital chain, but the further possibility of obtaining new data on some of the nervous processes soon became apparent.

We have described in other papers[2] the main phenomena of resuscitation of the central nervous system after cerebral anæmia. Here we shall briefly summarize the results of the resuscitation of the central nervous system as related particularly to the resuscitation of the entire animal body and incorporate at greater length some new observations on the more strictly physiological phase. Our work has dealt almost entirely with resuscitation after total anæmia except that, rarely, from some slip in technique or, more commonly, for some anatomical reason, the brain and upper cer-

[1] Received for publication April 16, 1908. The previous papers in this series have appeared in the *Journal of Experimental Medicine*, 1908, x, 371; the *American Journal of Physiology*, 1908, xxi, 359, and xxii, 51.

[2] Stewart, Guthrie, Burns and Pike, *Jour. of Exper. Med.*, 1906, viii, 289; Guthrie, Pike and Stewart, *Amer. Jour. of Physiol.*, 1906, xvii, 344; Stewart and Pike, *ibid.*, 1907, xix, 328; xx, 61; Stewart, *ibid.*, 1907, xx, 407.

vical cord still obtained a small amount of blood. Hill[3] states that "the merest dribble of blood" suffices to maintain a certain degree of activity of the central nervous system for long periods. To this we can fully subscribe. It is possible, also, that the mere presence of blood in the vessels, although completely stagnant, may help to prolong the period of resistance of the tissues to definitive death, so that it may not be indifferent whether the interference with the nutrition of the central nervous system is produced by occlusion of the whole arterial path to the central nervous system, as in most of our experiments, or by simple cessation of the circulation due to stoppage of the heart or, finally, by such a process as strangulation, which occludes simultaneously the arteries and veins. It is obvious that the greatest and most severe anæmia will be caused by the first method, a less complete and more gradual anæmia by the second, while the third will leave the normal amount of blood in the organ practically unchanged. The results of Ségalas d'Etchepare,[4] who found that the struggles of the animal persisted longer after ligation of the abdominal aorta when the inferior vena cava was also occluded than when the aorta alone was tied, would seem to indicate that stagnant blood is better than no blood at all in contact with the tissues. It is possible that such differences account, to a certain extent, for the discrepancies in the literature as to the length of time the circulation in the central nervous system can be stopped without rendering resuscitation impossible.

D'Halluin[5] states that the secondary alterations of the central nervous system following anæmia are not immediately fatal nor without remedy. He does not believe that Battelli's[6] limit of twenty minutes after cessation of the respiration is the absolute one for the resuscitation of the central nervous system, and is inclined to believe that there is experimental as well as clinical evidence that the central nervous system is capable of resuscitation after periods of perhaps relative but very prolonged anæmia. Green[7] states that in one of his cases, a boy nine years old, the

[3] Hill, *Philos. Transactions of the Royal Soc.*, 1900, cxciii, 121.

[4] Ségalas d'Etchepare, *Jour. de physiol. expér. et path.* de Magendie, 1824, iv, 287; cited by Batelli, *Jour. de physiol. et path. générale*, 1900, ii, 443.

[5] D'Halluin, *Presse méd.*, 1904, xii, 345.

[6] Battelli, *Jour. de physiol. et path. générale*, 1900, ii, 443.

[7] Green, *Lancet*, 1906, ii, 1708.

heart was not started by massage until twenty-five minutes after it first stopped, and did not beat strongly until thirty-five minutes after it first stopped. Severe spasms, rapid respiration (40) and pulse (168 in the minute), perspiration and high temperature followed, with death in twenty hours. This case might seem to lend some slight support to D'Halluin's conclusions. We have already pointed out in another place that the time of heart stoppage is impossible to determine except by actual inspection. For this reason we must exclude as evidence many of the clinical cases reported and cited in support of a longer limit, after which resuscitation is possible. We are not inclined, therefore, to give much weight to D'Halluin's contention.

Our experience, on the contrary, is that Mayer's[8] limit of ten to fifteen minutes of total cerebral anæmia, beyond which resuscitation is not possible, is approximately correct. This limit may be extended a little, but not a great deal, where the circulation has stopped completely without causing the extreme anæmia produced by our technique.

The exact moment at which the heart stops beating can be determined by inspection; and the last respiratory movement may easily be observed and recorded. Similarly, in the restoration of these systems to activity, the direct observation of the first movement is not a matter of great difficulty. The determination of the state of the cortical centers is somewhat different. There are, except in the case of the motor cortex in general, no unequivocal and objective signs of the loss or return of function.

In considering the resuscitation of the central nervous system we may most conveniently deal first with the return of the various functions and reflexes separately, considering them as criteria of the physiological condition of their respective centers. We will take up, therefore, the resusciation of the bulbar centers and then the return of the functions of the higher centers.

II. THE RESUSCITATION OF THE BULBAR NERVOUS MECHANISMS.

The Resuscitation of the Extrinsic Cardiac Nervous Mechanism.—The heart continues to beat in cerebral anæmia, apparently little

[8] Mayer, *Med. Cent.*, 1878, xvi, 579.

affected, long after the failure of all the cerebral centers, and even when the vagi, the cervical sympathetic, and the spinal cord are divided. Furthermore, a dog's heart will continue to beat for several hours after the removal of the head. It seems certain therefore, that the mammalian heart will beat without receiving any impulses from the brain. But will the heart beat continue indefinitely in the absence of cerebral impulses? So far, the only experimental answer to this question is that of Friedenthal[9] who severed all the cardiac nerves of rabbits, including the sympathetic, and all of the vagus except the Hering-Breuer fibers to the lungs. The animals lived for several months without suffering any great inconvenience during ordinary activities. We have observed some instances in the course of our experiment which would seem to be incompatible with Friedenthal's conclusions, but the question cannot be discussed here.

Asphyxial slowing of the heart may occur at any time during cerebral anæmia; and even when both vagi and the spinal cord are divided. The heart rhythm after restoration of the cerebral circulation, shown in detail in the tables in our previous papers, may indicate disturbances of the vagus center for several days afterward. Certain other influences, such as hyperthermia, may also enter in to affect the rate, and obscure the vagus effect. Quite apart from these, however, the vagus effect usually returns along with the function of the other bulbar centers, after which it may apparently fail for a time, perhaps several days, and then again finally recover its functional control of the heart.

The question of the nature of the origin of the vagus tone —whether reflex or automatic—has been discussed in a separate paper.[10] In the present paper we will offer some new evidence bearing on this question. We may, for example, investigate the relation between the first appearance of the vagus tone in the resuscitation period, and the first appearance of the vaso-motor tone. If the vagus tone is of automatic or central origin, we would expect a gradual decrease in the pulse rate as the blood pressure rises. As we have pointed out in the first paper of the

[9] Friedenthal, *Arch. f. Physiol.*, 1902, 135.
[10] Stewart and Pike, *Amer. Jour. of Physiol.*, 1907, xix, 328.

present series, the rate of the heart in the resuscitation period is that of a heart whose vagi have been divided, although the vagus endings in the heart are perfectly active. The heart maintains a practically constant, or even an increasingly high, rate as the blood pressure gradually rises. This evidence, as far as it goes, does not show the existence of an automatic element in the vagus tone. If now we investigate the time relations of the first appearance of vagus tone and the opening up of the reflex pathways for vagus inhibition, we find a very close correspondence in the time of their appearance—that is, there is little or no vagus tone discoverable in the resuscitation period until the integrity of the reflex arc is restored. There is, then, not only no evidence in favor of an automatic or central origin of the vagus tone, but also, as we have shown elsewhere, direct evidence in favor of its reflex origin.

Couty[11] states that the cord constitutes an independent cardiac accelerator mechanism as well as a vasomotor center, modified by anæmia, asphyxia and other influences. Couty used lycopodium spores, injected into the cerebral arteries, to produce anæmia of the brain. In all our experiments in which we were certain that the bulbar centers had ceased to function, we were unable to get the normal increase in heart rate by suddenly lowering the blood pressure. The heart beats on with machine-like regularity during the occlusion period. Barring the possibility of spinal shock in our experiments, we do not consider it probable that accelerator impulses normally arise from the cord below the fourth cervical segment—the lower level of total anæmia in most of our experiments, but we do not deny the possibility that a phylogenetically more primitive mechanism located in the cord, might, under proper conditions, again become active and discharge accelerator impulses to the heart.

One other line of evidence not previously considered remains to be examined, and that is the correspondence in the time of resuscitation of such a mechanism as that for swallowing, which is so evidently reflex, and the return of the vagus tone. But since, as is self evident, the same questions arise in connection with vasomotor tone and respiration, and the same standard of comparison may be

[11] Couty, *Arch. de physiol. norm. et path.*, 1876, Ser. 2, iii, 665.

used in determining their origin, we will first present the facts with regard to these mechanisms and then attempt their interpretation.

The Resuscitation of the Respiratory Nervous Mechanism.—It is obvious that, for the continued life of an animal the resuscitation of the respiratory mechanism is a matter of necessity. The heart may be beating vigorously and the tissues be well nourished so long as oxygen is provided by artificial means, but unless spontaneous respiration occurs, sooner or later death is inevitable. The choice of a method for starting the heart will therefore depend not only upon its efficiency in restoring cardiac function, but also upon the facility with which we may direct a stream of oxygenated blood through the medulla oblongata.

We have considered, in other papers, the resuscitation of the respiratory center after cerebral anæmia in much detail, and we have devoted particular attention to the question of the automaticity of the respiratory center.[12] The resuscitation of the respiratory center after asphyxia and other forms of death is very much the same as after cerebral anæmia provided the period of asphyxia has been reasonably long. There is, in general, the same strong first gasp, and the slow initial rhythm which gradually increases to the normal rate. The time required for recovery from transient respiratory failure due to anæsthesia is, of course, much shorter than that required for recovery from prolonged bulbar anæmia.

The regularity and constancy of the initial respiratory rhythm in resuscitation, three to five a minute, and the similarity of this rhythm to that sometimes observed after section of both vagi, and particularly after section of the higher pathways to the brain, is one argument we have advanced in favor of the automaticity of this center. The further fact that this slow, regular rhythm may persist for a time, the length of which depends partly upon the gravity of the injury to the cells, unaffected by stimulation of the afferent nerves is also an indication that the center is functioning in the absence of afferent impulses—*i. e.*, the center is automatic.

In a small dog (Experiment 1, March 13, 1905)[13] it was noted during the whole time of compression (three minutes and five seconds) of the arch of

[12] Stewart and Pike, *Amer. Jour. of Physiol.*, 1907, xix, 328.

[13] The experiments are numbered consecutively for convenience in reference. The chronological order is indicated by the dates given.

the aorta and of the innominate artery, that inspiratory acceleration of the heart occurred with each natural respiration, but not with artificial respiration. The impulses discharged from the respiratory to the cardiac center were not, therefore, reflex from the pulmonary fibers of the vagus. The quickening of the heart toward the end of the inspiration has been observed, in our later experiments, at a time when the afferent impulses did not affect the respiration.

Certain other structures participate in the respiratory activity by associated movements, notably among these being the eyelids.

For example, in Experiment 2, after an occlusion of sixteen minutes in a cat, the respiration was going on 34 minutes after the release at the rate of twelve in a minute. The movements were strong and extensive, the whole head participating in them. There were also strong fibrillary contractions of the tongue, and violent twitching movements of the vibrissæ. The pupils were well contracted, but there was no corneal, light or lid reflex. The animal winked its eyes slightly but distinctly, the upper eyelid descending with each respiratory movement. Again, in Experiment 3 (June 7, 1905), 28 minutes after an occlusion of 31 minutes, the pupils were well contracted, and the light reflex had returned, but not the corneal. The respirations at this time were rapid and in groups of four or five, getting progressively stronger up to a maximum. Strong nostril movements accompanied the respiratory movements. The eyelids moved with the strong respiratory gasps, the aperture of the eye widening, but not with the weak gasps.

The synchronism of the natural with the artificial respiration is a peculiar phenomenon seen in some of the experiments. An instance of this occurs in

Experiment 4 (March 29, 1905). Half grown cat. Ether. Tracheotomy. Artificial respiration. Opened chest.

1:40:15 P. M. Clamped aorta just above intercostal branches.

1:41. Clamped innominate and left subclavian arteries.

2:01. Released innominate and left subclavian arteries.

2:06. A gasp.

2:06:20. Another gasp. Gasping respiration goes on regularly, five in the minute.

2:11. No movements on striking forelimb.

2:12. Respiration strong and extensive, eight a minute.

2:14. Respiration 12 a minute, including some small respiratory movements one of which sometimes precedes a deep one. No movement elicited by striking the forelimb.

2:17. No light, lid, corneal, or ear reflexes. The respiration often commences with a rather prolonged, slight opening of the mouth, which ends in a wide gasp.

2:21. Pupil contracted down to a slit. No light reflex.

2:23. No eye or ear reflexes. No movements on pinching ear or on striking forelimbs. Temperature 33° C. in the thorax.

2:28. Swallowing movement.

2:30. The natural respiration is now synchronous with the artificial, ten in 18 seconds, extending to nose, mouth and chest.

2:33. On striking forepaw, get movement of same limb and very slight movement of the opposite shoulder.

2:34. No eye or ear reflexes. Pupils still slits. The natural respiration continues synchronous with the artificial.

2:37. On touching cornea, the eye seems to water, although the eyelids do not move. Marked movement of forelimb on striking it; also movement of opposite forelimb.

2:43. Fair corneal reflex. No light reflex. Strong movement of forelimb when struck and weaker but distinct movement of opposite forelimb.

2:45. Strong light causes marked closing of eyelids but no change in the pupil. Repeated many times.

2:50. Corneal reflex quite marked; also the foreleg reflex, which crosses from either side. Stopped artificial respiration. Cat breathes slowly by itself. Started artificial respiration again.

2:55. The forelimbs are extended and getting stiff. Light reflex is present in pupil.

3:00. Winks eye when ear is flicked. No ear reflex.

3:10. On pinching larynx, get a swallowing movement every time. (Stimulation of superior laryngeal?)

3:15. Stimulated left vago-sympathetic. Causes stoppage of heart, maximum dilation of pupil, bulging of eye and retraction of nictitating membrane.

At times the synchronism assumes a different form.

Thus, in Experiment 5 (March 21, 1905), beginning about twenty minutes after the head arteries were occluded, the cat inspired synchronously with the artificial inflation of the lungs, but only half as often. Twenty-two minutes from the beginning of the imperfect occlusion, natural expiration occurred synchronously with the inflation of the lungs, and exactly half as often. The occlusion lasted, in all, 30 minutes. Fourteen minutes after the release of the head arteries, the artificial respiration was interrupted, and the cat, which had been, for several minutes, breathing synchronously with the artificial respiration, went on breathing for 25 seconds with exactly the same rhythm.

Such instances are too common to be mere coincidences.

Apnœa during the resuscitation period is not uncommon. Instances of this have been given in previous papers. In one experiment, lowering the systemic blood pressure apparently again started respiration. Other cases in which hemorrhage or trephining the skull caused the apnœa to cease, *e. g.*, in Experiment 16, the protocol of which is given later on, have been encountered.

In Experiment 6 (May 13, 1905), three hours after release following an occlusion of one hour, stopping the artificial respiration caused respiratory movements to begin after a considerable interval. These movements involved, at first, the diaphragm alone and later on, the ribs also. Apnœa had lasted two hours.

Well-marked periodic respiration of the Cheyne-Stokes type sometimes occurs well on in the resuscitation period.

In Experiment 7 (May 2, 1905) the first gasp occurred fifteen minutes after a release from an occlusion of 30 minutes. The first gasps occurred at the rate of four in 64 seconds. About four hours after release, the respiration was 140 per minute, with strong associated movements of the nostrils. Pressing the fingers over the chest on each side of the sternum caused marked slowing of the respiration, persisting as long as the pressure was maintained. Twenty-five minutes later, repeating the pressure over the chest caused the same slowing of respiration. The respiratory rate was, at this time (7:38 P. M.), 100 a minute but there were marked variations in the rate at successive intervals of a few seconds. At 8:13, the hind end of the animal was elevated to allow fluid and mucus to run out of the mouth. The respiration decreased to 86 a minute for two or three minutes, but the rate soon rose again. At 8:18, the hind end was lowered again, and the respiratory rate fell to 84 a minute. Then, with the board in exactly the same position, the respiration rose to 100 a minute at 8:32, then to 144 at 8:38, to fall again to 106 at 8:46, and to 84 under the influence of a small amount of ether at 9:00 P. M. An hour later, the respiratory rate was 59 in the minute.

Movements closely approximating spinal respiration have also been observed. One example of this has been given in a previous paper,[14] in which we described also the reflexes simulating respiratory movements so readily obtained through the spinal cord in the resuscitation period following prolonged occlusions. One other case is of interest in this connection.

In Experiment 8 (May 22, 1905), two hours and forty-seven minutes after release following an occlusion of 60 minutes, abdominal movements began. Twenty-four minutes afterwards, on stopping artificial respiration, these movements spread upward to the thorax, producing fair ventilation of the lungs for three minutes, after which artificial respiration had to be started again as signs of asphyxia (stiffening of the forelimbs in extensor spasm) came on. The same series of events was repeated 46 minutes later. True bulbar respiration never returned.

This phenomenon has been observed so frequently that we may say, almost as a general rule, that in those cats which do not recover the power of normal permanent breathing of the bulbar type, imperfect respiratory movements seem to start from the lower part of the cord involving the hind legs and abdomen and then, if sufficiently strong, extending up to the thorax.

The Resuscitation of the Bulbar Vasomotor Mechanism.—We

[14] Stewart and Pike, *Amer. Jour. of Physiol.*, 1907, xix, 339.

have given in another place[15] the physical requirements, in the matter of a suitable blood pressure, for the continued activity of the heart. Still more striking examples are given by Goltz.[16] In dogs whose spinal cord had been transected in the lower dorsal region and allowed to recover, destruction of the cord below the level of the transection was followed by death in thirty hours or less. According to Goltz the cause of death was the low blood pressure.

It is clear, from a consideration of these facts, that a certain blood pressure is necessary for the continued action of the heart and respiratory mechanism. So long as the peripheral vessels remain widely dilated, and the blood passes through with relatively little resistance, this condition will not be fulfilled. The arterioles must be constricted to their normal diameter, the splanchnic vessels must give up their enormous volume of stagnant blood, and the natural resistance to the blood flow be reëstablished. All this is a part of the work of the vasomotor mechanism. Before an animal can be considered as resuscitated, we must, therefore, restore the system or center which maintains the necessary blood pressure. In selecting a method for starting the heart, we must keep in mind that it is necessary to maintain, or very soon to establish, a circulation through the respiratory and vasomotor centers, particularly through the latter. If a blood pressure sufficient for the activity of the heart can be maintained, the oxygenation of the blood can easily be accomplished. The maintenance of a sufficient blood pressure by any means which is not in itself injurious, or which does not curtail the flow of blood through the vasomotor and respiratory centers is, however, far from easy of accomplishment. Aside from the resuscitation of the cortical centers, to be discussed in a subsequent section, we may regard the restoration of the vasomotor center as the completion of the final link in the vital chain.

The Relative Resistances of the Vasomotor and Respiratory Centers to Cerebral Anæmia.—The exact moment at which the vasomotor center ceases to function is not so easy to determine as the time at which the respiratory center fails, as there is no such

[15] Pike, Guthrie and Stewart, *Jour. of Exper. Med.*, 1908, x, 371.
[16] Goltz, *Archiv für die gesammte Physiologie*, 1873, viii, 460.

sudden cessation of a very noticeable phenomenon connected with the former as with the latter mechanism. Instead of a last great movement, the vasomotor center fails gradually, and the time of cessation of function is further masked by the fact that there are accessory vasomotor centers in some animals which assume a part of the function of the medullary center on the failure of the latter. It is our opinion that the vasomotor center is more resistant to anæmia than the respiratory center, and attains a certain degree of functional activity after the restoration of the cerebral circulation before the respiratory center gives any sign of its activity. In this condition, we must remember that a part of the vasomotor mechanism is not subjected to anæmia, while no accessory respiratory centers concerned in the first movements in the resuscitation period exist outside the medulla oblongata. We are therefore restoring only a part of a system in the first case and a whole system in the second. In order to make conditions strictly comparable, we must destroy the function of all vasomotor centers as well. This is accomplished in asphyxia or any other form of general death. Exact observations, aside from Hayem's[17] work on death from hemorrhage, are not at hand in sufficient numbers to decide unequivocally which center persists longer in general death, but we believe that the vasomotor center is the more resistant, and that it is also somewhat easier to restore to a certain degree of functional activity than the former center. In cerebral anæmia, with all the systemic vessels, aside from those of the head open, Traube-Hering curves have sometimes appeared in the blood pressure tracing after total cessation of respiratory movements. In these experiments, it must be assumed that the vasomotor center is the more tenacious of life.

We have previously discussed the condition of the vasomotor centers in the spinal cord, and will quote only one new experiment here.

The pads on the hind foot of a cat, when placed in contact with ice, became very red at a time in the resuscitation period when no eye reflexes had returned and when the bulbar vasomotor center had, in all probability, not recovered its tone. The reaction was brought about, therefore, either through the vasomotors of the cord, or through a local mechanism.

[17] Hayem, *Arch. de physiol. norm. et path.*, 1888, Ser. 5, i, 103.

Although the spinal cord centers, or what we have supposed to be spinal vasomotor centers, maintain a fair blood pressure in the absence of the cerebral centers, unequivocal reflexes are not easy to obtain through the former, at least in the occlusion period and early in resuscitation. There seems to be a marked automatic element in their activity, but further work on this point is necessary. It is possible that this absence of spinal vasomotor reflexes, as we will point out later, may be due to spinal shock.

The tonicity of the bulbar vasomotor mechanism begins to return, in the resuscitation period, as a rule before the respiratory center begins to discharge, and, as is the case with the respiratory center, there is often a period during which the blood pressure may be rising, but when reflex changes in pressure do not occur through stimulation of the vagus or brachial nerves. Similarly, a time may often be found in the occlusion period when the blood pressure is still high, and the bulbar vasomotor mechanism undoubtedly functional, when stimulation of the afferent nerves produces no effect on the blood-pressure curve. We have attributed the persistence of pressure after the cessation of the reflex effects, and this early rise in pressure before the opening up of the reflex pathways to the automaticity of the nervous mechanism involved.

TABLE I.

Showing the length of the occlusion period and the time of return of deglutition, as compared with the return of respiration.

Date.	Length of Occlusion.	Time after Release of First Gasp.	Time after Release of First Swallowing Movement.
March 3, '05	6 minutes	40 seconds	3½ minutes
March 18, '05	12 min. 45 sec. Heart stopped 15 to 20 min. Started by massage.	Less than 38 min.	52 minutes
March 29, '05	10 minutes	5 min. 40 sec.	14½ minutes. Several movements.
March 29, '05	20 minutes	5 minutes	27 minutes
June 7, '05	31 minutes	9 minutes	6 hours

Considered either from the view point of resistance during the occlusion period, or the return of function during the resuscitation period, the respiratory and vasomotor mechanisms show a greater vitality than either the extrinsic cardiac or the swallowing mechanisms.

The Resuscitation of the Swallowing Mechanism.—The resuscitation of this mechanism is of peculiar interest from the fact that swallowing is so clearly a reflex act. The time in the resuscitation period at which the first swallowing movement was observed is given in Table I. In all experiments the respiration returned a considerable time before swallowing was observed. In all experiments, too, the blood pressure had risen to a considerable height. Swallowing has, however, been observed before the return of the eye, ear or fore-limb reflexes. Experiment 4 may be cited as a case in point. After long occlusions, swallowing has been observed to return along with the rolling movements of the eyes and the secretion of tears a considerable time after the restoration of the cerebral circulation, *e. g.*, in one experiment, six hours after an occlusion of thirty-one minutes.

When the order of disappearance of the reflexes and functions of the bulbar centers is studied, it will be noted that swallowing may persist longer than the corneal or light reflexes, but ceases before the end of the secondary series of respiratory gasps or the failure of the vasomotor center. The time relations of the cessation of some of these functions are shown in the following protocol.

Experiment 9.—(April 19, 1905.) Cat. Ether. Tube in larynx.

3:12 P. M. Occluded head arteries.

3:12:15. Corneal reflex gone. Respiration stopped, pupils dilated, legs straightened out. Started artificial respiration.

3:12:25. Swallowed.

3:12:48. A slight respiratory movement.

3:13:05. Another slight respiratory movement.

3:13:15. Pupil about one half maximal dilation. Several strong swallowing movements.

3:13:40. Pupils now much wider, nearly maximum. A strong gasp occurred.

3:14:05. Several strong gasps in quick succession, and kicking movements of hind limbs.

3:15. Struggling of whole body, except head. Tail lashing violently for a long time.

3:15:30. Strong struggling movements. Strong lashing of tail, and scratching movements of hind limbs.

3:16:05. All movements stopped. There have been no respiratory movements for a minute.

3:18. Pulse 258 in the minute.

3:19:20. Hind limbs now quite limp. *No response on pulling or striking.*

3:22. Put thermometer into rectum. Hips twitching when thermometer was passed. Rhythmical contraction of rectal and vaginal sphincters going on. Temperature 36½° C.

3:25:30. Moving thermometer in rectum causes contraction of vaginal and rectal sphincters and whole perineum.

3:30:15. Released head arteries.

3:42. Good kicking movements of hind legs on putting thermometer into rectum. Rhythmical movements of rectal sphincter, about twelve a minute.

In this experiment, the persistence of the swallowing mechanism is intermediate between that of the eye reflexes and of the bulbar respiratory and cardiac mechanisms. The same time relations appear in many other experiments, and we have never observed the persistence of swallowing after the respiration had permanently ceased, or the blood pressure fallen very low in the occlusion period.

The activity of the swallowing center, as we have shown in another place, may be conserved for a time when other fluids are substituted for blood.

The effect of the fresh defibrinated blood, artificially circulated through the head of a dog, in maintaining the irritability of the œsophagus and the integrity of the nerve endings in the tongue, and upon the submaxillary glands and the sympathetic endings in the pupil as well, is shown in the following experiment.

Experiment 10.—(February 28, 1905.) Medium sized dog. Ether.

2:40 P. M. Drew off 830 c.c. blood. Began artificial respiration.

3:15. Tied aorta and put cannula in the central end. Heart soon stopped.

3:26. After elevating hind end and bandaging abdomen, tied inferior vena cava and put cannula in heart end. Tied subclavian arteries and veins.

3:29. Tied a ligature around heart in auriculo-ventricular groove, leaving out inferior and superior cavæ. No eye reflexes.

3:30. Started artificial circulation with a double bottle arrangement. The blood would not run out of the cannula in inferior cava, although the auricle and superior cava and inferior cava down to the cannula were full. Removed clot. Blood now runs off.

3:40. Circulation was really started.

3:48. Stimulated left vago-sympathetic, in continuity, without tying. Good bulging of the eye and dilation of pupil. Secretion, or possibly liquid gastric contents, started to flow from the nose. There were a few drops before, but it is now much increased. The pupils have narrowed considerably since the artificial circulation started.

3:50. Stimulated right vago-sympathetic, in continuity, without tying. Marked bulging of the eye, opening of palpebral fissure, and dilation of pupil.

3:59. Still considerable liquid dropping from nose. Good bulging of eyes on stimulation of the vago-sympathetics.

4:02. Opening of left eye and dilation of the pupil on stimulation of vago-sympathetic. Immediately after stimulation much liquid runs out of mouth and nose, evidently from the œsophagus, and contains small granules of food.

Either the œsophagus contained liquid, which stimulation of the vagus caused it to expel, by causing contraction, or vomiting was caused from the stomach by vagus stimulation. The mucous membrane of the mouth and tongue is moist and well flushed with blood. Vaso-dilation seems to be present.

4:23. Marked dilation of pupil and bulging of right eye on stimulation of right vago-sympathetic. Exposed left chorda tympani and Wharton's duct. The duct is empty. Stimulation of chorda does not cause filling of duct.

4:35. Stimulated each vago-sympathetic. Marked opening of eyes but now little or no dilation of pupil. Stimulated central end of left lingual nerve. No reflex movements.

4:43. Stimulation of left vago-sympathetic causes marked bulging of eye, some retraction of nictitating membrane and slight dilation of pupil.

4:46. Stimulation of left hypoglossal nerve causes strong contraction of tongue. Motor endings intact.

4:53. Trephined and stimulated motor area on right side. No effects of any kind. No eye movements nor change in pupil on left side. No movements of neck nor left forelimb. The jaws are now showing some rigor (decerebrate rigidity?). Stimulated left vago-sympathetic. Fair dilation of pupil.

4:55. Again stimulated right motor cortex. No effect even with strong stimulation. Cut out gray matter and stimulated corona radiata. No effect. Stimulated axillary nerves on left side. Good contraction of muscles. Direct stimulation also caused good contraction. Stimulated œsophagus. Good contraction. The œsophagus seems to retain its excitability better than anything else in the neck. Stimulated left sciatic nerve. No effect either direct or reflex. Stimulated hind leg muscles directly; no effect.

On post mortem, found air bubbles in vessels at base of brain, *e. g.*, basilar, and also on cortex.

All through this experiment from the time the artificial circulation was properly started, the flow of blood was good and the carotids showed a good pressure. The blood was in an air-pressure bottle under about 100 mm. Hg.

It will be noted that the peripheral nerves and muscles, and particularly the œsophagus, retained their irritability for a considerable time after the motor cortex became inexcitable. The motor endings in the tongue were also fairly well conserved by the artificial circulation.

The œsophagus remains irritable, both to direct stimulation and to stimulation of the vago-sympathetic, for long periods of time under the influence of artificial circulating fluids. Stimulation of the vago-sympathetic may also affect the œsophagus at a time when there is no effect on the eye, although the eyelid responds readily to direct stimulation. This difference in the reaction of the two structures may be due to the interpolation of a synapse on the path to the eye, while there is, so far as we are aware, no synapse on

the path to the œsophagus. Instances of such phenomena have been given elsewhere. In one experiment, Number 11 (March 1, 1905), the œsophagus about two inches above the heart was observed to beat with almost the same rhythm as the auricles. We do not know the cause of these rhythmical contractions, but offer the suggestion that they might have been due to the action current of the heart. As is well known, the action current of the heart may, under certain conditions, excite the phrenic nerve and cause rhythmical contractions of the diaphragm which are synchronous with the heart beats.[18]

The Comparative Argument for the Automaticity of the Respiratory Mechanism.—Additional evidence of the automatic or reflex nature of the origin of cardio-inhibitory and vasomotor tone and of the respiratory movements, as we mentioned above, may be obtained from a comparative study of these mechanisms with that for deglutition. We have pointed out elsewhere that the synapse is a weak link in the nervous reflex arc. So far as we can see, there is no apparent reason why two such arcs as that through the respiratory center and that through the swallowing center should not be resuscitated at approximately the same time. As Marckwald[19] has shown, there is a very intimate physiological, and probably anatomical, connection between these two centers. If respiration were reflex, as swallowing so evidently is, we should expect a very close correspondence in the time of resuscitation of these two mechanisms. But when we look for such correspondence in time, we do not find it. On the contrary, as will appear from the various protocols quoted and from Table II, we find that respiration returns considerably earlier in the resuscitation than swallowing does. The most obvious conclusion is that respiration occurs in the absence of afferent impulses. The time at which stimulation of the afferent nerves begins to affect respiration corresponds much more closely with the time at which swallowing first occurs.

The vasomotor and swallowing mechanisms may be compared in a similar way, and again we conclude, from the early appearance of vasomotor tone, and the relatively late return of the influence of

[18] Stewart and Pike, *Amer. Jour. of Physiol.*, 1907, xix, 339.
[19] Marckwald, *Zeit. f. Biol.*, 1888, xxv, 1.

afferent nerves upon the blood pressure, that there is a very marked automatic element in the origin of the vasomotor tone. Very probably the circulation of the blood to the different organs according to their needs is brought about reflexly, but it seems to us that the height of the general systemic blood pressure may well be due to an automatic mechanism.

When we compare the time of the resuscitation of the cardiac centers with that of the swallowing centers, the results, and therefore the conclusions also, are somewhat different. The respiration becomes established, and the blood pressure reaches a considerable height—perhaps its maximum, before reflex cardiac effects appear. Then, suddenly, the reflex arc is again integrated and the heart becomes visibly affected by afferent impulses to the bulbar centers. In point of time, cardio-inhibition appears much nearer the return of swallowing than do the other two functions. We conclude, therefore, that cardio-inhibition and acceleration are very largely reflex in origin.

When we study the relative order of failure of these four functions during the occlusion period, we find further evidence in favor of these conclusions. The march of events is so rapid, and certain other conditions, to be mentioned presently, which enter in here, complicate things so much that this evidence is probably not so trustworthy as that drawn from observations during the period of resuscitation. The swallowing movements fail first, then the cardiac impulses and finally the respiration and vasomotor tone. We might reasonably expect therefore that, since the synapse is the weak link, the reflexes would fail first, and the automatic functions persist longer.

The only disturbing factor is that entering into the deportment of the heart, since cardiac acceleration persists longer than we would expect a pure reflex to do. It is possible, but perhaps not probable, that the mere asphyxiation of the cells of origin of the inhibitory nerves serves to excite them until they succumb, at which time the accelerators become active, as we have previously pointed out.[20] It is, however, noteworthy that even the cardio-acceleration fails before respiration, and it has not been shown that the cardiac

[20] Stewart and Pike, *Amer. Jour. of Physiol.*, 1907, xix, 349.

centers ordinarily remain active when afferent impulses are excluded, without, at the same time, interfering with the afferent paths.

Bearing in mind all of the phenomena of occlusion and resuscitation, we conclude that respiration and vasomotor tone are, at their inception in the resuscitation period, almost or quite automatic; later, afferent impulses begin to affect them; the cardiac centers, on the contrary, are, under normal conditions, almost or quite dependent upon afferent impulses for their activity.

III. THE RESUSCITATION OF THE HIGHER NERVOUS MECHANISMS.

The Return of the Eye Reflexes.—The relative time of the failure of the eye reflexes, as compared with the respiration, during the occlusion period, and of their return in the resuscitation period have been given in detail in another paper, and will not be dwelt upon at length here. There are, however, certain new facts in the action of the cervical sympathetic upon the pupil that we wish to present in this paper.

The artificial circulation of fluids through the head does not, as a rule, long maintain the integrity of the sympathetic endings about the pupil. This is shown in the various protocols already cited in the first of these studies. Again, the cervical sympathetic, when stimulated, may fail to affect the pupil, but the lachrymal gland will secrete, apparently in response to the stimulation.

In one experiment (12) in which the head arteries were occluded for several short periods, and a mixture of fresh defibrinated blood and Locke's solution circulated through the head later, stimulation of the left vago-sympathetic nerve, in continuity, caused enormous dilation of the left pupil, with bulging of the eye and retraction of the nictitating membrane. At the end of one minute, the left pupil was still widely dilated, and the right pupil was also dilated, although not as much as the left. Twenty-five minutes later, stimulation of the left vago-sympathetic gave the same effect on the left eye as before. After an interval of a few seconds, the right pupil also enlarged, the lateral half dilating much more than the medial half. Two minutes later, the left vago-sympathetic was again stimulated. Some dilation occurred in the left pupil, which had remained much dilated since the previous stimulation, but there was no effect on the right pupil, which had remained the same size. Very shortly afterward—three or four minutes—both pupils became totally insensible to stimulation of the cervical sympathetic.

From the protocols given in the first of these studies, we see that the œsophagus may respond to stimulation of the vago-sympathetic trunk in continuity after the pupil fails to respond, although the pupil may still respond to direct stimulation in some instances.

In Experiment 13 (April 3, 1905) the left pupil remained much narrower than the right for a long time in the resuscitation period. Three and one fourth hours after release, following an occlusion of 50 minutes, neither section nor stimulation of the left vago-sympathetic caused any change in the left pupil, which remained contracted. The right pupil was at maximal dilation.

We have supposed there might be a synapse on the sympathetic pathway to the eye, which broke down, under the adverse influences of anæmia and the artificially circulated fluids, sooner than a direct pathway free from synapses.

We have observed, also, that, when the eyes are widely opened and bulging, and the cornea hard, probably indicating a high intra-ocular pressure, the stimulation of the cervical sympathetic may not cause maximum dilation of the pupil, and that whatever dilation occurs disappears soon after the cessation of stimulation, the pupil quickly returning to normal.

There has been much discussion among authors as to whether the respiratory or the pupillo-motor center is the more resistant to cerebral anæmia, and which was the first to be resuscitated. Hayem[21] states definitely that the respiratory center is the last of the cerebral centers to fail in death from hemorrhage. We have found that the respiration returns in cats before the pupils are contracted. Herlitzka,[22] in discussing some of D'Halluin's[23] criteria of resuscitation, states that contraction of the pupil may be a purely hydraulic phenomenon[24] depending upon pressure factors alone, and is not, therefore, an accurate index of the functional activity of the pupillo-motor center. In the absence of all other cerebral functions the contraction or dilation of the pupil may not be a trustworthy sign of functional activity, but if other centers are active, we see no reason why the state of contraction of the pupils should

[21] Hayem, *loc. cit.*

[22] Herlitzka, *Arch. ital. de biol.*, 1905, xliv, 93.

[23] D'Halluin, *loc. cit.*

[24] Mosso, cited by Herlitzka, *loc. cit.*

not be regarded as a valid index of the functional activity of the pupillo-motor center. Indeed, we have experimental evidence that the state of contraction of the pupil does not always depend upon blood pressure alone. We have pointed out, in our first paper, the difficulty of distinguishing between the effects due to the cerebral center and those due to sympathetic influences and will not enlarge upon them here.

The mere contraction of the pupil, as it occurs in the return of cerebral function, must be carefully distinguished from the contraction and relaxation due to changes in the intensity of the light. The pupils may be contracted in the resuscitation period, and the light reflex absent. During the succeeding days the pupils may be wide, especially during the spasms, and the light reflex present, in some degree at least.

The widening of the pupils during spasms, as we have stated elsewhere, is an almost, or even quite, constant occurrence. The dilation may not appear as soon as the spasms begin, but some hours after, and is always plainly evident in those animals which are kept alive on the day following the operation.

In Experiment 14 (May 13, 1905), for example, in which the head arteries were occluded for one hour, permanent extensor spasms of the forelimbs were noticed five hours and 46 minutes after release of the head vessels. Fairly strong spasms of the forelimbs began six hours after release, and increased in severity as time elapsed. It was not until eight hours and 20 minutes after release that the pupils were observed to dilate during the spasms, which, at that time, were of considerable severity.

We have mentioned in another place the fact that the cornea becomes sunken and furrowed during the period of anæmia, if the occlusion is at all prolonged, and that the cornea may again become smooth and firm in the resuscitation period. The time in minutes after occlusion at which the corneal tension begins to decrease, and the time in minutes after release when the cornea again becomes firm, are shown in Table II, which gives also the length of the

TABLE II.

Length of Period of Occlusion in Minutes.	Minutes after Occlusion when Tension Begins to Lessen.	Minutes after Restoration of Circulation when Tension Returns.
31	3 min. 50 sec.	21 min. 30 sec.
10	7 min. cornea furrowed	18 min. 30 sec.
15	———	32 min.
8	30 sec.	5 min. 40 sec.

occlusion period. The effect of intraocular tension upon the response of the pupil to stimulation of the cervical sympathetic has already been mentioned.

The intra-ocular tension may be maintained for a time, or even restored in rare instances, by a good circulation of fresh defibrinated blood through the head end of an animal.

The Temperature of the Animals in the Post-Anæmic Period.—The question arises whether the temperature of the animal remains the same or whether the heat regulating mechanism, sharing the general derangement and sometimes apparent hyperactivity of the other nervous mechanisms, maintains an abnormally high temperature for a time.

At the conclusion of the operation, and after the respiration has returned so that the animal is able to breathe spontaneously, the temperature, taken in the rectum, is often subnormal. The temperature as measured at varying intervals during the post-anæmic period is given in Table III.

The temperature immediately before occlusion of the cerebral arteries was sometimes subnormal, *e. g.*, 36° C., particularly if the dissection of the vessels had been tedious, but was always markedly below normal at the end of occlusion, falling to 32° C. in one instance. A fall of 0.6° C. occurred during an occlusion of ten minutes. In the cases where marked cerebral injury resulted from the occlusion, the temperature often fell one or two degrees after the release of the cerebral arteries, but subsequently rose again. In a case of severe convulsions the temperature was 39.7° C. on the day after the operation. In another case where no violent convulsions were observed after the first few hours, the temperature was 32° on the day after the operation. Another animal which recovered without abnormal symptoms had a temperature of 38.4° C. on the second day. In some instances where the temperature fell very low, the animal was put in a covered box which could be heated by a burner beneath it. Sometimes a rise of temperature followed this procedure, the rise continuing after extinguishing the flame or removing the animal from the box, and reaching a point somewhat above normal. Putting in the ice chest, primarily for the purpose of controlling the spasms, caused a fall of temperature,

TABLE III.

Showing the length of the occlusion period, and the temperature of the animals at varying periods after the release of the cerebral arteries.

Number of Experiment.	Length of Occlusion.	Temperature (Rectal).	Time after Release.
I.	60 minutes 30 seconds	32° C.	4 min.
		31	24 "
		30.5	37 "
		30.4	47 "
		30	1 hr.
	Artificial heat	29.8	1 " 27 "
		29.5	1 " 42 "
		30	2 "
		30.30	2 " 8 "
		31.2	2 " 19 "
		32.8	2 " 43 "
	No more heat	33.5	2 " 58 "
		33.8	3 " 33 "
	Artificial heat	33.5	5 " 45 "
		33.5	5 " 58 "
	Burner turned out	33.5	6 " 4 "
		34.2	6 " 58 "
		34.4	7 " 6 "
		Put in ice chest	
		32	7 " 33 "
	Not on ice	32	7 " 47 "
		32.2	8 " 5 "
	In ice chest		
		30.4	8 " 38 "
		30.4	8 " 56 "
		30.4	9 " 8 "
		30.7	9 " 33 "
		32.4	12 " 23 "
		Death	15 " 8 "
II.	26 minutes		
		33.3	5 "
		32	6 "
		33.5	21 "
		33	34 "
		31	4 " 37 "
		32	20 " 50 "
		32.9	26 " 20 "
III.	10 minutes 7 seconds	35.4	During occlusion
		35.2	5 "
		35	14 "
		34	1 " 24 "
		39.7	21 " 24 "
IV.			
		34	30 "
		37	20 "
V.	45 minutes		
		36.5	At release
		36	14 "
		35.3	26 "
		34.8	38 "
		34.6	51 "
		34.3	1 " 5 "
		33.4	1 " 53 "
		33.3	2 " 54 "

the fall sometimes continuing after the removal from the ice. In general, the temperature regulating mechanism of the animal suffers along with the other cerebral mechanisms, the extent of the variations of the temperature depending, in a somewhat general way, upon the severity of the general effects of anæmia. If the animal recovers its other cerebral functions the temperature regulation, as a rule, again becomes normal.

Von Bechterew[25] and others were led, from a consideration of clinical evidence, to suspect the existence of a cortical center for the secretion of sweat. In a recent paper, von Bechterew[26] gives the experimental evidence for the existence of such a center. On the basis of this work we may explain the cause of the sweating in Green's[27] case by supposing that the center for the secretion of sweat, like many other cortical centers, is not acting in a normal manner, or may even be hyperexcitable, as the cortical motor centers apparently are. The conditions in cats are not so easy of observation, and we have no constant results on this phase of the subject. In most instances, in which attention was directed to this point, the pads of the feet were moist.

Some glycosuria is always present in animals which live more than a few hours. This is not due to the anæsthetic alone, as was shown by control experiments. It is possible that the injury or excitation of the medullary "diabetic center," similar to that which produces puncture glycosuria, may explain the appearance of dextrose in the urine. The experiments of von Bechterew showed that stimulation of a particular area of the motor cortex in the dog caused an increased flow of urine, and, frequently also, glycosuria. The derangement of such a cortical mechanism in the cat would explain the glycosuria so constantly noted.

The Resuscitation of the Cerebral Motor Cortex.—The activity of the motor cortex is not well maintained by artificial circulation of defibrinated blood or other fluids, as is shown by the protocols of the experiments already quoted. Moreover, the white matter, *e. g.*, the fibers of the corona radiata, soon loses its excitability

[25] v. Bechterew, *Arch. f. Physiol.*, 1905, 297.
[26] v. Bechterew, *loc. cit.*
[27] Green, *loc. cit.*

under the influence of the artificial circulatory fluids, as shown in Experiment 10. The cortex is inexcitable during the period of anæmia, and may fail to recover after the normal circulation is reëstablished.

In Experiment 15 (March 4, 1905) the head arteries of an adult cat were occluded for ten minutes. The pupils slowly contracted after release of the head vessels, but began to dilate again about one hour after release. No corneal or other reflex ever returned. Shortly after the two pupils began to dilate (62 minutes after release) about 100 c.c. of a mixture of fresh defibrinated blood and Locke's solution (45 c.c. blood diluted to 275 c.c.) was injected into the aorta under a pressure of 100 mm. of mercury to increase the volume of circulating fluid. The pupils became narrow in nine minutes after the injection was begun. Three minutes later, stimulation of the left vago-sympathetic produced dilation of the left pupil, retraction of the nictitating membrane and bulging of the eye. The dilation of the pupil disappeared almost as soon as the stimulation was stopped, but the bulging of the eye persisted a little time. Eighteen minutes after starting the injection, gasping movements occurred, increased in strength and persisted thirteen minutes. At the end of this interval, 31 minutes after starting the injection, the left pupil was dilating and the blood pressure in the carotid was low. About 30 c.c. of the blood mixture was now injected, and respiratory movements came back almost at once, but grew feebler in four minutes. Marked spasms of the face muscles occurred. Thirty-six minutes after starting the first injection a third injection of the blood mixture was begun. Artificial respiration was suspended for 40 seconds, and then started again. The respiratory gasps began almost immediately afterward. At this time, 41 minutes after the first injection, the animal puckered up its face and closed its eyes tightly. The pupils dilated greatly, and both respiratory gasps and facial spasms ceased in one and one half minutes. A fourth injection was begun 65 minutes after the first. The pupils again contracted somewhat, only to dilate again, and nasal and lachrymal secretion appeared. A fifth injection occurred 72 minutes after the first, and again the pupils contracted. At this time, stimulation of the left vago-sympathetic caused dilation of the pupil. Eighty-one minutes from the beginning of the first injection, the skull was trephined and the left motor center stimulated. There were no movements of any kind. The cortex was then excised and the corona radiata stimulated, with the same result as for the cortex. Seven minutes later, the experiment was stopped. There was some twitching of the right eyebrow at this time, and the heart was still in good condition.

In Experiment 16, a part of the protocol of which is here cited, the motor cortex was excitable to direct stimulation fifty minutes after starting the heart.

Experiment 16 (March 18, 1905). Cat. Ether. Tube in larynx, artificial respiration.

11:28 A. M. Occluded head arteries.

11:40:45. Released head arteries. Soon after this, animal stopped breathing. Opened thorax and massaged heart directly.

12:05 P. M. Heart started again.

12:57. Pupils well contracted. No light reflex but corneal reflex is present.

12:57:30. Light reflex back. Swallowing. Marked lachrymal secretion.

1:02. Pupils of normal size. No reaction of animal to loud sounds produced by clapping hands. Shoulders and head move when board is sharply struck.

1:09. Touching interior of ear causes movements of eyelid on same side. These are produced especially well by flicking the ear with the finger. No movements of eyelids on opposite side.

1:22. Moved head on whistling in ear but not on blowing into it, though blowing caused lid movements.

1:45. Put ammonia in the nostrils. No effect. Put ammonia in the mouth. Strong movement of head and neck and some movements of forelimbs. Trephined skull.

1:55. Stimulated motor cortex. Got jaw movements of opposite sides. No other movements except perhaps slight movement of opposite forelimb. As soon as the skull was opened, occasioning some hemorrhage, deep regular spontaneous respiratory movements began, involving head, neck and chest down to diaphragm. It was not observed whether the diaphragm took part. These movements were much more effective for respiration than the previous gasping respiratory movements.

Similar results have been obtained by Scheven[28] on rabbits. This observer found that the white matter of the central nervous system was more resistant to anæmia than the gray matter. Stefani and Cavazzani[29] found that the rabbits' peripheral nerves would retain their conductivity for afferent impulses up to ten minutes after being deprived of their blood supply.

We have previously pointed out[30] that the convulsions occurring during the resuscitation period after cerebral anæmia[31] are not necessarily due to the motor cortex, since the same phenomena occur in animals in which the brain has been divided at about the level of the roots of origin of the fifth nerve.

Micturition sometimes occurs during these convulsions, with the anatomical connections intact. While it is possible that the expulsion of the vesical contents is due to the spasm of the abdominal muscles, there is also the possibility of involvement of the cortical motor center for the bladder.

[28] Scheven, *Arch. f. Psychiatrie,* 1904, xxxviii, 926; *ibid.,* 1904, xxxix, 169.

[29] Stefani and Cavazzani, *Mem. letta all' academia medico-chirurg. di Ferrara,* 1888, cited by Scheven.

[30] Stewart and Pike, *Amer. Jour. of Physiol.,* 1907, xx, 72.

[31] Stewart, Guthrie, Burns and Pike, *Jour. of Exper. Med.,* 1906, viii, 307.

Mewing, indicating recovery of the cortical area for phonation, has been observed twenty-two and one half minutes after release from an occlusion of five minutes. The light reflex was well marked at this time, and the pupils were of normal size. The spasms were marked. The mournful mewing was evidence of a considerable degree of cortical activity. In another experiment, of fifteen minutes occlusion, the spasms were of marked severity six hours after release. The cat uttered a peculiar "yowl" from time to time during the spasms, and particularly during the latter stage of a spasm. This cry may have been from the corpora quadrigemina,[32] and not from the cortex. The cat was found dead fourteen hours later.

Consciousness, in some degree, has been observed twenty-five minutes after release from an occlusion of nine and five sixths minutes. There was no light reflex present at this time, but the tail was wagging. Flicking the fore paws caused strong gasping movements. Consciousness, and the reflexes have persisted throughout an imperfect occlusion of eighteen and one half minutes, followed, thirty-five minutes later, by a second imperfect occlusion of thirty minutes. The fore limbs, particularly the left, were weak until the animal's death eight days later, and straddled out from the body in walking. A spastic gait is often observed when the animals begin to walk about after recovery from the operation.

Voluntary movements of the head, neck, shoulders and fore limbs, but no corneal reflex, and respiratory movements involving the muscles down to the shoulders, have been observed eight minutes after release from an occlusion of six minutes. Voluntary change of position was observed in another animal three and one half hours after release from an occlusion of thirty-one minutes. In another case, no voluntary movements ever returned, even twelve hours after release from an occlusion of forty-five minutes, although the circulation to the head was good all of the time.

Continuous sleep, with tightly closed eyes, was observed twenty hours after release from an occlusion of five and five sixths minutes following an incomplete occlusion of nine and one half minutes.

[32] Ferrier, Functions of the Brain, 2d edition, New York, 1886, p. 166.

The rectal temperature was 38.7° C. The clonic spasms were entirely gone, and did not appear on stimulation. The respiration was thirty-three a minute, and regular. The light and corneal reflexes were well marked. The animal swallowed salt solution placed in the mouth, and tickling the ear caused the ordinary movements of it.

The Senses after Recovery from Anæmia.—Sight is often apparently normal after cerebral anæmia, but apparent total blindness has also been met with. Blindness did not occur immediately, but developed within a week or ten days after occlusion. Strangely enough, the light reflex appeared to be present in some degree. The corneal reflex still remained, for the eyelids were closed when the eyeball was touched. The pupils were often wide and staring, but were sometimes seen to be narrowed in bright light. The location of the lesion, whether in the retina or in the cerebral center, and its nature are unknown.

We have observed wide and staring pupils in animals whose sense of sight was still present. The nature of the impression made on the disordered brain is a matter of conjecture. The animals howled when anyone came into view, but showed neither fear nor affection. The vision often becomes apparently normal so far as we were able to determine by our tests. In one experiment, the sense of sight had evidently returned three hours and fifty minutes after release from an occlusion of nine minutes and fifty seconds.

Hearing.—Like sight, the auditory sensation shows varying degrees of recovery. After anæmia and during the period of convulsions, there is often apparently increased sensitiveness to slight sounds. The ticking of a watch is often sufficient to cause convulsions, all other stimuli being excluded. In favorable cases, the return of hearing is apparently complete. Total deafness, as a result of cerebral anæmia is rather doubtful, but deafness to ordinary sounds is well established, for example, calling, and the mewing of kittens were unnoticed. A loud, sharp report near the ear usually attracted some attention. The location and nature of the lesion are unknown.

Hearing returns somewhat earlier in the resuscitation period

than sight. In one experiment, in which the heart was started by massage after a stoppage of fifteen to twenty minutes, loud sounds caused no reaction one hour after the heart began to beat. One hour and twenty minutes after starting the heart, the head moved on whistling in the ear, but not on blowing into it, although this blowing caused eye movements. Again in another experiment in which the heart stopped for about ten minutes after an occlusion of six minutes, the cat moved on striking the board sharply fifty minutes after starting the heart, but not on calling or clapping the hands. Hearing had probably not returned in this last experiment.

The recognition of sounds returns somewhat later than the mere audition. In the last experiment in the preceding section (in which there was an occlusion of nine and five sixths minutes), the animal recognized sounds, *e. g.*, "pussy," three hours and twenty minutes after the release of the head arteries. Thirty minutes later there was evidence of sight. In another experiment, the sense of hearing was apparently abnormally acute fifty minutes after release after an occlusion of three and one third minutes, the animal starting at any sound, and going into tonic extensor spasms. Two hours later, the cat was sitting in the normal position of a couchant cat, but with the head turned sharply to the right. A loud sound caused it to change its position, the animal lying on its belly with its head straight in front.

The results on *taste* and *smell* are not so definite. Ordinarily the animals eat well, and manifest a preference for certain kinds of food. Food may be taken when the animal is blind and deaf. Milk held near the nose may not be lapped unless the nose is actually touched with it, but, once tasted, is eagerly eaten.

Touch.—After the exaggerated sensibility to touch of the first few hours or days following anæmia, the sense of touch becomes apparently normal in animals which show otherwise favorable symptoms. There is the normal response to stroking the fur. Even a blind and deaf cat showed this response and would often mew when stroked. We believe it is a response to touch as a sensation rather than the response of a spinal animal to a stimulus. The reflex response to tickling the ears was always observed if a

sufficient time was allowed to elapse after anæmia. Pain and temperature sensations were present in the cats which recovered well otherwise, but the responses to these stimuli could not always be interpreted in animals which suffered apparent loss of some of the other senses.

The Return of the Mental Processes.—This is perhaps the most interesting of all the phases of the resuscitation of the brain, and a phase which can be studied in detail by the clinical observer alone. Animals may be watched in the laboratory, and may manifest all the functions normal to the ordinary animal, but we are unable to tell whether the mental processes are completely restored.

So far as we are able to judge from the deportment of the animals, all the mental faculties may return after short periods of occlusion (eight minutes). The animals observed showed no noticeable change in deportment. The responses to calling and stroking were normal. A white rat allowed to run in front of one cat was at once pounced upon, and was rescued only with difficulty. On the application of a non-irritating antiseptic solution to the skin lesions which developed later, the cat showed the usual aversion to water. The general results in this case were decidedly encouraging. We have other examples of apparently complete return of the mental processes after occlusions of six minutes, seven and one half minutes, nine and five sixths minutes and sixteen and one half minutes. In this last experiment, however, we doubt the totality of the anæmia produced.

Such a favorable result does not always follow. After an occlusion of ten minutes, we have seen apparently the total loss of intelligence. We have also observed an apparent insanity along with the return of the bodily functions.

In a previous paper we pointed out that strychnine, either had no noticeable effect on the previously anæmic cord or else paralyzed it, when injected in very small quantities, before affecting the normal cord. In looking over our experiments, we find that only one cat survived the subcutaneous injection of strychnine, the rest all dying within a few hours at most. The action of strychnine in excess is to paralyze the normal cord. Since the previously anæmic area may reasonably be supposed to have a lowered vitality

and a diminished resistance to external influences, it is possible, and, in the light of our experiments, very probable that the therapeutic dose of strychnine may have an injurious effect instead of a beneficial one.

The Pilo-motor Mechanism.—Although it is probably not to be considered as exclusively under the control of the higher centers, the pilo-motor mechanism may be discussed here. Movements of the hair of the cheeks, and particularly of the vibrissæ, are not infrequent in the resuscitation period.

For example, in Experiment 17 (May 27, 1905), 45 minutes after release, following an occlusion of 45 minutes, there was constant twitching of the hair of the throat and cheeks, and of the vibrissæ. At this time the respiratory gasps were occurring at intervals of 18 seconds, and there were clonic movements of the forelimbs, with a response to striking or pinching the limb on the same side stimulated. The forelimb reflexes did not cross.

In Experiment 18, nearly five hours from the release of an occlusion of 51 minutes, the hairs on the tail were erected one minute after stopping artificial respiration (natural respiration had not returned). A second short period of asphyxia three minutes later was followed by the same result. Erection of the hairs on the tail and back has been observed during the spasms. In Experiment 14, the hairs of the tail and back were erected continuously (indicating tonic pilo-motor spasms) eight hours and 40 minutes after release from an occlusion of 60 minutes. At this time the pupils were beginning to dilate during the spasms.

CONCLUSIONS.

The experiments on cerebral anæmia have enabled us to duplicate, by an entirely different method, many of the results obtained by anatomical division or removal of parts of the central nervous system. In some respects the method of anæmia permits of greater precision than the method of division or excision, and avoids, in great measure, the disturbances due to the wound and to the hemorrhage caused by the latter method. The method of general anæmia, as Couty[38] pointed out long ago, leaves something to be desired in the matter of exact localization, but this objection may be met, in some degree at least, by appropriate methods of investigation. It is desirable that the results obtained by the method of section should be duplicated by some other method in order to eliminate as much as possible the effects due to the irritation produced by the anatomical lesion.

[38] Couty, *loc. cit.*, p. 754.

Our results show, as we believe, that, of the bulbar mechanisms studied, the respiratory is the most automatic, the vasomotor in part automatic, and the cardiac like the swallowing mechanism, almost wholly dependent upon afferent impulses for the arousal and discharge of its normal activity.

The eye reflexes return during the resuscitation period in the animals in which the cerebral anæmia has not been too prolonged. The motor cortex loses its excitability during anæmia, but may regain it after the reëstablishment of the circulation. The pilomotor mechanism is disturbed during the spasms which occur at a certain stage in the resuscitation. The temperature falls during the occlusion period, but rises again, often to far above normal, in the days following the anæmia. So many disturbing factors, such as the violent muscular contractions during spasms, enter into the problem that it is impossible to say that there is an actual disturbance of the temperature regulating mechanism although we are inclined to believe that this is the case.

All the senses return, following cerebral anæmia, but sight and hearing may afterwards fail without causing the death of the animal. The mental processes may return without any apparent deficiency, if the period of anæmia has been short. After longer occlusion, apparent insanity has been seen, and in one case, apparent total loss of mental processes occurred.

The reflex excitability of the cord returns rather early in the resuscitation period. Reflexes from the anterior part of the cord first involve muscles on the same side as the stimulus, and later cross to involve muscles of the opposite side. The spinal cord sometimes falls into much the same condition as that following spinal transection, and the scratch reflex appears. Spinal transection, when these reflexes have appeared, does not produce shock. Practically all phenomena of spinal shock may be reproduced without section of the cord. We conclude, therefore, that spinal shock is due more to the cutting off of the reflex pathways through the higher centers of the nervous system than to the stimulation of inhibitory fibers by the anæmia.[84]

[84] Pike, Guthrie and Stewart, *Amer. Jour. of Physiol.*, 1908, xxi, 371.

THE RATIO BETWEEN THE HEART-WEIGHT AND BODY-WEIGHT IN VARIOUS ANIMALS.[1]

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Introduction.—In this paper are given the results of observations, which I have made, on the relation between the heart-weight and the body-weight in different animals. So far as I am aware, very little, if anything, has appeared on this subject in this country, although considerable work has been published on this and closely related subjects in Europe. For that reason I have thought it worth while to collect from previous investigators a few of the figures which seemed most interesting and have arranged them in tabular form near the end of this communication.

In the literature, we find numerous tabulations which give the weight, dimensions, etc., of the various organs of the body, and also the relation between the size of individual organs and the size of the body. These have been determined for man in the normal and in several pathological conditions. Vierordt (1) gives tables showing the weights and measurements of almost every organ of the body. Bergmann (2) gives the relation of the heart-weight to the body-weight in man and several species of animals. Parrot (3) published an extensive article upon this relation in fifty or more species of birds. Hasenfeld and Romberg, (4) W. Müller (5) and Külbs (6) may be mentioned. Many of the normal ratios were determined in order to have controls for experiments upon the effects of exercise or valvular defects of the heart upon cardiac hypertrophy and related subjects. There are some variations between the figures of these investigators for an individual species, but for the most part they are fairly constant. The use of different methods may account for many of the differences.

[1] Received for publication May 6, 1908.

The Writer's Observations.—During the past five months the relation between the heart-weight and body-weight has been studied in the animals used in this laboratory for other purposes. The animals were weighed before any operation was begun, after death the heart was removed in a uniform manner (by cutting all vessels at the point where they joined the heart), the cavities were opened and carefully washed free from clots and the heart was weighed. The animals in which this relation was studied were dogs, cats, rabbits and guinea-pigs.

By the term "ratio of heart-weight to body-weight"—a term which will be used frequently in this paper—is meant the number of grams of heart tissue for every kilogram of body-weight. For example, a ratio of 4.5 would mean four and one half grams of heart tissue per kilo of body-weight.

The ages of the animals in my series of observations were unknown. No comparison, therefore, could be attempted between the ratios of younger animals and those that were older.

The animals of each species have been grouped in two ways. *First,* they were grouped according to *sex,* in order to get a comparison between the average ratio of heart-weight to body-weight in males and females of an individual species. *Second,* they were grouped according to *sex* and *body-weight.* To do this the animals of the first grouping were arranged so that the average ratio of animals having an equal (or nearly equal) body-weight could be determined. The purpose of this was to compare the ratios of lighter animals with those of heavier animals of the same species.

Grouping According to Sex.—In the following table there is an arrangement of animals according to sex only. Column six contains the "ratios."

TABLE I.

Species.	Sex.	Number of Animals.	Average Body-Weight in Grams.	Average Heart-Weight in Grams.	Average Ratio in Grams.	Average Per Cent. of Heart-Weight to Body-Weight.
Dog	Male	58	8,029	59.23	7.43	0.743
	Female	60	6,038	45.46	7.61	0.761
Cat	Male	11	3,078	13.80	4.56	0.456
	Female	15	2,650	12.34	4.58	0.458
Rabbit	Male	38	1,606	4.31	2.67	0.267
	Female	66	1,697	4.57	2.70	0.270
Guinea-pig	Male	14	384	1.70	4.22	0.422
	Female	33	257	1.03	3.91	0.391

It will be seen from the table that the ratios for cats and guinea-pigs are almost the same, while that for dogs is much higher and that for rabbits much lower. As a possible explanation for the very low ratio of the rabbit, two factors may be mentioned. First, the inactivity of tame rabbits and probably a larger amount of body fat as a result. Second, the large size of the stomach and cæcum. Especially after a very full feeding this last factor would exert a marked influence upon the ratio. A ratio derived from rabbits with the weight of stomach and cæcum contents excluded would undoubtedly be nearer that found in the other animals.

The difference in the ratios of males and females in an individual species is so slight in my series as to be of small significance. In cats and rabbits the male and female ratio is almost identical. In dogs the table above shows a difference of only .18 gram of heart-tissue per kilogram of body-weight in favor of the female. In guinea-pigs there is a difference of .31 gram per kilogram in favor of the male.

In December a preliminary report upon the ratios of dogs and rabbits was made before the New York Pathological Society. In that report the tables contained half or less than half the number of animals which are included at present. It is interesting to note that after almost doubling the number of these animals, the ratios have scarcely been changed at all. This fact seems to indicate that the ratios given represent the true ratio for each species. It is illustrated in the following table, which contains the number of animals upon which report was made at the meeting in December and also the number in the present report, with the corresponding ratios.

TABLE II.

Species.	Sex.	Table Referred To.	Number of Animals.	Ratios.
Dogs.	Male.	Former report.	28	7.50
		Present "	58	7.43
	Female.	Former "	25	7.64
		Present "	60	7.61
Rabbits.	Male.	Former "	21	2.70
		Present "	38	2.67
	Female.	Former "	36	2.88
		Present "	66	2.70

From this table we see first that the addition of thirty male dogs changed the ratio only .07 grm. of heart-tissue per kilogram of body-weight; second, that the addition of thirty-five female dogs changed the ratio only .03 grm. per kilo; third, for male rabbits the addition of seventeen made a change of .03 grm.; and fourth, for female rabbits, the addition of thirty made a change of .18 grm. per kilo. This last is by far the largest variation of the four, and yet even it is, for practical purposes, negligible.

In the following table are given the highest and lowest ratios found in each sex of each species. The difference between them is quite marked in some instances, especially in the cases of male dogs and male guinea-pigs. However, there were very few animals of these two species which gave such a wide variation from the average obtained for the species.

TABLE III.

Species.	Sex.	Highest Ratio Found.	Lowest Ratio Found.	Average Ratios Given in Table I.
Dog	Male	10 55	5.35	7.43
	Female	9.51	5.72	7.61
Cat	Male	5 86	3.61	4.56
	Female	5.57	3.35	4.58
Rabbit	Male	3.42	2.07	2.67
	Female	4.47	2.00	2.70
Guinea-pig	Male	7.03	2.64	4.22
	Female	5.79	2.66	3.91

Heinz (7) (Vol. I, Part 2, page 886) gives a table of nineteen dogs with an average ratio of 9.71. Among the nineteen dogs he has seven with a ratio of 10.00 or over and eight with a ratio of 9.00 or over. In my table, with a total of 118 male and female dogs, there is only one animal which goes as high as 10.00 (and that one was a very distinct case of pathological hypertrophy), and but six animals as high as 9.00. My average for dogs, it will be remembered (Table I), was about 7.50—or 2.2 grams per kilo dog less than the average given by Heinz. That is, taking a ten kilo dog, the total heart-weight, using Heinz' average, would be 97.0 grams, while a dog of the same weight, using my average, would have a heart weighing but 75.0 grams—a difference of 22 grams. This is a much greater difference in weight than could be explained by the

use of a different method of removing the heart. It would suggest a possible difference between the dogs of Germany and of the United States, or rather of New York.

Groupings According to Sex and Weight.—In the following table the dogs have been arranged in groups according to their body-weight, those having an equal, or almost equal, weight being placed in a single group.

TABLE IV.

Body-Weight.	Female Dogs.		Male Dogs.	
	Number of Animals.	Average Ratio.	Number of Animals.	Average Ratio.
3,000– 3,999	6	8.07	1	9.69
4,000– 4,999	13	7.88	4	7.58
5,000– 5,999	12	7.90	7	6.16
6,000– 6,999	10	7.28	8	7.82
7,000– 7,999	9	7.13	13	6.99
8,000– 8,999	6	7.13	7	8.18
9,000– 9,999	3	7.48	7	7.18
10,000–10,999			2	7.23
11,000–11,999			2	7.13
12,000–12,999			4	7.22
13,000–13,999			2	6.45

We see from this table that in the case of both male and female dogs, as we pass from the lighter to the heavier ones, the ratio gradually becomes less. Or, in other words, the relation of heart-weight to body-weight follows, in general, the law of an inverse proportion. It seems to me this law might be connected with the well-known observation that the smaller the animal the higher the pulse rate. We know that skeletal muscle responds to increased activity by a hypertrophy. Since a more rapid heart beat means greater activity of cardiac muscle, may it not be that this faster rate of beat in the younger animal is the cause of a physiological hypertrophy of the heart?

When the average normal relation between the heart-weight and body-weight has once been determined, it is found that, in general, this average ratio holds fairly constant for members of that species. This is illustrated by the following instance: A dog, whose body-weight had not been previously determined, was used in the laboratory. After death, the body-weight was estimated from the weight of the heart (using an average for dogs already determined) to be

12,450 grams. To test the accuracy of this estimate, the animal was then weighed and due allowance made for loss of blood during the operation. The actual weight was found to be 12,100 grams, an error of only 350 grams. There were many more instances in which a like result was obtained by a reverse of the above illustration, *i. e.,* by estimating the heart-weight from the known body-weight, then weighing the heart to find how accurate the estimate had been. It was a very common thing to predict within one to three grams the total heart-weight.

There are, however, several factors which tend to cause individual variations from a general average ratio determined for a species. These factors may be placed under two heads. First, those which cause changes in the size of the body, such as growth (normal or abnormal), excess of fatty tissue, pregnancy, emaciation, etc.; second, those which cause changes in the size of the heart, such as increased bodily activity or pathological cardiac conditions from which compensatory hypertrophy of the heart would result.

Vierordt has shown in his published tables that for man the relative amount of heart-tissue per kilo of body-weight decreases with the advance of age, or, in other words, that the *young* individual has a higher ratio than the older individual. It is found in other tables (my own included) that the *light* individuals have a higher ratio than heavier members of the same species. It would seem that both these factors, *i. e.,* age and size, exert a certain influence upon the ratio. Of course, we usually expect a young animal to have a smaller body-weight than an older one, and if this were invariably true, it would be unnecessary to speak of age and size as separate factors. But we know that these two factors need not, necessarily, run parallel, since we have young animals of large size and older animals of small size.

Another thing which may cause a difference between the ratios of two animals of the same species, independently of a change in the size of the heart, is the presence or absence of a normal amount of fatty tissue. For instance, an emaciated dog, because of a reduction in the body-weight, would show a high heart ratio, while for an opposite reason, a very fat dog would show a low ratio. In predicting the size of the heart in a live animal, it is sometimes nec-

essary to keep this fact in mind. Aside from the three factors already mentioned, the literature contains abundant evidence that increased bodily activity or valvular defects of the heart will bring about very great differences in the ratios of an individual species. For example, Külbs took two young dogs from the same litter and kept one of them shut up in a cage, while the other was made to exercise strenuously in a treading machine. After some time they were both killed. The body-weight of each was almost exactly the same, but the heart of the exercised animal weighed 152 grams, while that of the caged animal weighed only 99 grams. This experiment gives one a definite idea of the pronounced effect which the factor of bodily exercise may have upon the size of the heart.

Whether the amount of muscular activity can account altogether for the difference in the ratios of the *different species* of animals seems somewhat doubtful, and yet this factor undoubtedly exerts a marked influence, as is shown by the following table of ratios, collected from different sources. The animals are so arranged that

TABLE V.

Species.	Sex.	Body-Weight in Grams.	Heart-Weight in Grams.	Grams of Heart Tissue, per Kilo of Body-Weight.	Author Quoted.
Tame rabbit	Unknown	1,916	4.56	2.36	Hasenfeld und Romberg
Wild rabbit	Unknown	1,120	3.07	2.76	Grober[8]
Guinea-pig	Male	384	1.70	4.22	Joseph
	Female	257	1.03	3.91	"
Pig	Unknown	49,700	225	4.52	Bergmann
Cat	Male	3,078	13.8	4.56	Joseph
	Female	2,650	12.3	4.58	"
Cattle	Male	280,000	1,450	5.35	Bergmann
	Male (cst.)	545,000	1,410	3.86	"
	Female	398,000	1,523	3.83	"
Man	Male	58,000	340	5.88	"
	Female	50,000	273	5.47	"
Sheep	Male	20,600	127	6.17	"
	Female	20,600	118	5.85	"
Horse	Male (cst.)	493,000	3,000	6.77	"
	Unknown	417,000	2,400	5.81	"
Hare	Male	3,666	28	7.70	"
	Female	3,500	25	7.17	"
Dog (American)	Male	8,029	59.23	7.43	Joseph
	Female	6,038	45.46	7.61	"
Dog (German)	Unknown	4,706	47.3	9.71	Heinz
Deer	Male	20,600	238	11.55	Bergmann
Bird (thrush)	Unknown			25.64	Parrot

the one with the lowest ratio is at the top and that with the highest ratio at the bottom of the list, the column of ratios (Column 5) exhibiting a gradual increase as one approaches the bottom. It is also very plain that the amount of bodily activity in the various animals given follows, in a general way, the same order of increase as the ratios—the more active animals being at the bottom of the table.

One or two points shown in this table are worthy of special notice. We find the tame and wild rabbits at the top with ratios of 2.36 and 2.76 respectively. Now if we look near the bottom of the list, we will find the hare, a very near relative of the rabbit, with a ratio of almost 8.0. In this case it is very easy to see the influence of activity.

The ratio of the deer (11.55) and especially of the thrush, a very active flyer and songster (25.64)—almost twelve times as great as that of the tame rabbit—are also very interesting.

It gives me pleasure to acknowledge the helpful suggestions of Dr. Meltzer given during the preparation of this paper.

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THE PRODUCTION OF AGGLUTININS IN THE ANIMAL BODY BY THE INOCULATION OF SUBSTANCES OTHER THAN PRODUCTS OF BACTERIAL ORIGIN.[1]

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There is to-day among observers working along the lines of immunity a tendency to no longer consider a given anti-body and the factors producing it as a compact inseparable entity following the laws of protoplasmic continuity, but as a complex body that may be split up into component parts, which may exhibit individuality of structure and independence of action. This view of the subject has been given an impetus by the work of Vaughan and later by Obermeyer and Pick. Vaughan derived split products from bacterial proteids and egg-albumen, representing a poisonous and non-poisonous portion. Obermeyer and Pick found by iodizing protein they so changed it that when the iodized portion was inoculated into animals only non-specific precipitins were formed. This led them to believe that specificity in this case was due to the aromatic radical which was changed by iodization.

The following work was begun in 1905 with hope of determining through the nature of substances having the power to produce agglutinins in the animal a more intimate knowledge of the antibodies and their elaboration in the animal economy. This in a measure was realized. As the work progressed the fact developed that, as far as tested, certain molecules or radicals containing such molecules, are always present in the substances, which induce an increase of agglutinin production; this fact strongly suggests the possibility that these molecules are responsible, for one feature at least, of this phenomenon. The observations arrange themselves under the following heads: (1) Organized ferments, (2) unor-

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ganized ferments, (3) metabolic products, (4) putrefactive products, (5) inorganic substances.

Organized Ferments.—Ballner and Sagasser in 1904 succeeded in producing, artificially, agglutinins for *Bacillus typhosus* by inoculating animals with red yeast cells (Rosa hefe), but the yeast cells themselves were not agglutinated by the serum. Acting upon this suggestion of Ballner, I inoculated rabbits with living cultures of brewer's yeast. After four or five inoculations the sera of these rabbits were tested with the following organisms:

1. *B. dysenteriæ,* 3 strains (Shiga, Flexner Manila, Park Mt. Desert).
2. *B. typhosus,* 2 strains (Pfeiffer, Mt. Sinai).
3. *B. Coli,* 2 strains (Laboratory, Colon X).
4. *B. paratyphosus.*
5. *B. pyocyaneus.*
6. *B. proteus vulgaris.*
7. *S. choleræ.*
8. *Pneumococcus,* one strain.
9. *Streptococcus,* one strain.
10. *B. mallei.*

The result of inoculations with yeast cell may be shown in the serum of a young goat by the following table.

TABLE I.

	Normal Serum			After 8 Inoculations.					
	10	20	50	10	20	50	100	200	500
Shiga	—	—		—	—				
Flexner Manila	+	+	—	++	++	++	++		
Park Mt. Desert	—	—		—	—	—			
Pfeiffer	+	—		++	++	++	++		
Colon	+	—		—	—				

	After 13 Inoculations.						After 18 Inoculations.						
	10	20	50	100	200	300	10	20	50	100	200	500	1000
Shiga	—						—						
Flexner Manila	++	++	++	++	++	+	++	++	++	++	++	++	++
Park Mt. Desert		—	—	—	—		—	—					
Pfeiffer	+	+					—						
Colon	—						—						

Several rabbits inoculated with yeast cells also gave the same results as the goat. The serum of these rabbits when tested showed a steady increase after inoculation, for the Flexner Manila strain of *B. dysenteriæ,* an increase for *B. typhosus* and sometimes for

Colon X strain of *B. coli* with the subsequent disappearance of the two latter. This disappearance of the agglutinins for *B. typhosus* and *B. coli* would seem to indicate one of two things. First, the normal cells of the rabbit possess a greater potentiality for the manufacture of Flexner Manila agglutinins, on account of the cell being subjected to a stronger and more specific stimulation in this direction and therefore when influenced by non-specific substances the cell forms agglutinins for this organism in preference to others because of this stronger initial stimulation. As the inoculations proceed the increment of production for the Flexner Manila strain becomes more marked and finally the entire energies of the cell seem to be occupied in forming these agglutinins to the exclusion of the others.

On the other hand the possibility suggests itself that the stimulating agent may not be as wholly non-specific as generally considered; this phase would then agree with that seen when definite specific organized agents such as bacteria or their products are used, stimulation of common agglutinins being induced. Common agglutinins may continue throughout to be formed to an equal degree along with the specific, or may disappear almost entirely after long immunization. This disappearance or persistence seems to depend somewhat upon the relation of the heterologous organisms to the homologous. The more nearly the species are related the more persistent the common agglutinins.

I have not been able to identify the Rosa hefe used by Ballner and Sagasser. The red yeast mentioned in most works on fermentation is, strictly speaking, a torula and not a true yeast. Several rabbits were inoculated with the cells of the so-called red yeast obtained from the air. This did not give rise to an increase in the production of agglutinins, though later these same rabbits responded to inoculation of brewer's yeast cells. Several strains of brewer's yeast, differing in their action upon beerwort, were tested without presenting any appreciable variation in results. The brewer's yeasts were obtained in pure cultures from the laboratory of the Brewer's Academy, New York City, and were cultivated on 10 per cent. beerwort agar. Living cells were used. Beerwort alone inoculated into rabbits was without effect.

The serum of the young goat mentioned above was subjected to the exhaustion test with *B. typhosus,* the Flexner Manila organism and living brewer's yeast cells—and cells of the torula. The agglutinins for Flexner Manila were completely exhausted by this organism. *B. typhosus* and the yeast cells absorbed all but five per cent. of the Flexner Manila agglutinins. This amount not absorbed approximately represents the amount of normal agglutinins present in the serum, which on account of its more specific nature would resist the action of purely non-specific agents.

Unorganized Ferments.—The possibility that other enzymes might bring about this increase in the animal of preëxisting agglutinins suggested the use of the unorganized ferments, diastase, pancreatin and invertin. The results obtained by the inoculation of these substances coincide with those obtained by the inoculation of yeast cells.

The agglutinins were in each case increased from 1 : 50 to 1 : 500 after five or six, weekly, inoculations. The enzymes, however, failed to absorb the agglutinins thus raised from the homologous serum.

Products of Metabolism.—Nuclein as a component part of the yeast cell was first used. Vaughan obtained a certain amount of protection against the pneumococcus in guinea pigs by the inoculation of nuclein previous to the pneumococcus infection. The nuclein was used in the form of a nucleoproteid from the pancreas, for which I am indebted to Dr. Levene of the Rockefeller Institute for Medical Research. The other products used of the metabolic group were lecithin and proteoses from egg. The same increase of agglutinins for Flexner Manila type of *B. dysenteriæ* followed the inoculation of these substances as with yeast and enzymes. There was the initial rise for Flexner Manila type, *B. typhosus* and *B. coli,* with the subsequent dropping out of the two latter and a continued rise of the former as the inoculations were continued.

Products of Putrefaction.—The products of putrefaction tested were indol, skatol, ethyl mercaptan and phenol. I am indebted to Dr. C. A. Herter for the indol, skatol and mercaptan, and also for some valuable suggestions as to their use.

Ethyl mercaptan in one per cent. solution produced an increase of agglutinins in two rabbits after the fifth inoculation and followed the same course as in the cases where the enzymes and metabolic products were used. A control rabbit on injection of ethyl alcohol did not show an increased production of its normal agglutinins. Indol and skatol were without effects, as I expected, on account of the readiness with which they combined with the preformed sulphates and were excreted. Phenol, however, did enter into systemic relation with the organism and toxic effects were demonstrated in the animals inoculated, but a rise of agglutinins did not occur after a number of inoculations, the animals finally dying from excessive abscess formation.

At this point two factors present themselves as possible influences in causing an increase in agglutinins. The first is the increased production and destruction of leucocytes and the second is the fact that the substances bringing about this increase of agglutinins with the exception of the enzymes, concerning whose structure little is known, all possess a formula containing phosphorus or sulphur molecules, while those failing to effect an increase do not possess either element.

First the effect of the inoculations of these substances upon the leucocytes may be considered. Dieudonné claims that animals possessing agglutinins for a certain organism may have the amount of agglutinins increased by the inoculation of exciters of leucocytes such as aleuronat and hetol. Aleuronat in our hands brought about an increase of the initial agglutinins similar to that caused by the substances used in the preceding experiments. Hetol was not used.

To test the effect of our inoculations upon leucocytes, blood counts were made upon normal rabbits, rabbits inoculated with substances increasing the agglutinins, and substances which did not affect the agglutinins. The blood counts exhibit the same irregularity in the normal rabbit and in the inoculated rabbits irrespective of the occurrence or non-occurrence of an increase in agglutinins. This irregularity is in accordance with the statement of Brinckerhoff that the number of leucocytes per millimeter in the peripheral blood of the rabbit is constantly changing. It would appear from this irregularity that leucocytes could not account for the increased

production. The action of aleuronat should, therefore, be ascribed to its behavior as a proteid or derivative of a proteid rather than as an excitor of leucocytosis.

Inorganic Salts.—To follow more in detail the suggestion developed by the fact that phosphorus and sulphur were the elements possessed in common by the augmentors of agglutinins, several soluble inorganic salts of sulphur and phosphorus were tested, and their action was controlled as far as practical by salts containing the same base but not the same radical.

Several rabbits were inoculated with sodium phosphate, sodium sulphate, calcium and potassium phosphate. Control rabbits were inoculated with sodium chloride, calcium chloride, and potassium chlorate. A young goat was inoculated with sodium sulphate and a control goat with sodium chloride. The sera of the rabbits and the goat receiving the sulphur and phosphorus compounds showed the characteristic increase of agglutinins for the Flexner Manila strain of *B. dysenteriæ*. The agglutinins for *B. typhosus* and *B. coli* were only slightly stimulated in a few instances. The agglutinins of the control animals inoculated with the salts that did not contain the sulphur or phosphorus molecules were unaffected. The doses varied according to the toxicity of the salts used and the concentrations ranged from one tenth normal to twenty-five per cent. solutions. The concentration of the solutions had no apparent effect upon the results. It is interesting to note that Vaughan found the phosphorus in the non-toxic portion of the split products which is the part that gives immunity.

A question arises as to the character of the action of these substances. Do they merely stimulate to greater activity a specific function of the cell already established or do they possess something in common with the bacteria which admits of their initiating specific action in some degree? An attempt was made to answer this question in the following manner.

Two rabbits each having normal agglutinins for the Flexner Manila type of *B. dysenteriæ* up to 1 : 50, but none that were appreciable for Shiga or Park Mt. Desert types, were inoculated one with the Shiga type and the other with the Park Mt. Desert organism. After three inoculations the sera of these rabbits aggluti-

nated their homologous organisms in dilutions of 1 : 100. The index for the Flexner Manila type remained unchanged. The bacterial inoculations were then stopped and sodium sulphate substituted in one rabbit and diastase in the other. After four or five treatments the sera from both rabbits were tested. The agglutinins for the Flexner Manila organism were increased up to 1 : 500, while those for Shiga and Park Mt. Desert type remained unchanged and later disappeared.

These two experiments are not sufficient, however, to prove or disprove the assumption that the action is one of augmentation and not of initiation. I have mentioned the foregoing question because it suggests several points to be taken into consideration in answering it. First, the influence that has brought about the normal agglutinins in the rabbit for Flexner Manila has been acting practically during the adult life of the animal, while the influence of the Shiga or Park Mt. Desert organisms has only been exerted for a comparatively short period of time; hence the function of the cell to form the Flexner Manila agglutinins would be more permanent than for the other two organisms. Now when the rabbits are inoculated with strong specific substances as Shiga or Park Mt. Desert the cell responds accordingly as long as this stimulus is kept up—but upon removal of this influence and the substitution of a, presumably, non-specific stimulus, then the cell by preference responds in the direction of the more accustomed function of forming agglutinins for Flexner Manila. Another point to be considered is the fact that after withdrawal of the specific influences of Shiga and Park Mt. Desert the cause producing the normal agglutinins for Flexner Manila in the rabbit continues to act, thus adding its influence to the stimulus of the non-specific sodium sulphate inoculations and so determining the direction of the activity of the cells.

At this stage of the present investigation biological rather than physical laws seem to offer the most probable explanation of the production or augmentation of agglutinins which has been described; perhaps stimulation of certain cell activities occurs because some necessary element which enters into the cell or acts as a ferment adjuvant is provided.

The work of several authors on ferments is suggestive by anal-

ogy. Bertrand found that manganese was present in laccase and activity of laccase was proportional to the amount of this salt present.

Calcium salts are found to be essential to enzymes which cause clotting and Magnus has given the name of co-ferment to those substances, but this term has been rejected by Harden and Young. Harden and Young found that boiled yeast which was incapable of initiating fermentation alone when added to unboiled yeast increased its action to a considerable extent. They found this increase due to the presence of arsenates which brought about the same results as the phosphates.

It is too early to draw definite conclusions concerning the effect of various substances upon the production of agglutinins and the suggestions that have been offered are tentative, pending further work which is in progress.

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SOME DIFFERENTIAL COUNTS OF THE CELLS IN THE LYMPH OF THE DOG: THEIR BEARING ON PROBLEMS IN HÆMATOLOGY.[1]

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Our knowledge of the cell-formula of the lymph is nearly comprised in the statement that small mononuclear elements make it up almost entirely. No systematic differential counts of the lymph-cells in man or in other animals are on record. According to the most recent work, that of Weidenreich (1), whose paper is as yet only reported in brief, one finds in the thoracic duct of the rabbit, the dog, the cat, the guinea-pig and the monkey, non-granular cells in large number, "especially little lymphocytes, but next to these large leucocytes with round nuclei, in which last all stages of mitosis are met. . . . Finely granular leucocytes (neutrophile or amphophile) are few, as are eosinophile leucocytes."

As an aside to another investigation I have had opportunity to observe cells from the lymph of a number of healthy dogs. Differential counts of these have disclosed facts which seem worthy of report.

There exists a good general description of the elements found in the lymph of the dog—that of Biedl and v. Decastello (2). They noted in the fluid all the cells of the animal's blood except the mast-cell. Polymorphonuclear neutrophiles were present only as evidence of blood-contamination. Mitoses in the mononuclear leucocytes were rare. Once they found a large number of eosinophile cells independent of blood-admixture. These authors give no differential counts of the leucocytes. Delamere (3) mentions that out of 133 successive cells he noted 128 lymphocytes, three polymorphonuclear neutrophiles, and one eosinophile.

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Lymph for the counts here recorded was obtained by operation on animals anæsthetized with morphine and chloroform. A fine, glass cannula was inserted in the thoracic duct near its mouth, and cover-glass preparations immediately taken, to be colored later with Wright's stain. The counts, which for the most part total five hundred cells—never less than three hundred—were in each case derived from observations on several slips, made from different drops of lymph collected at short intervals. In this way an attempt was made to obtain the average cell-formula, but the individual spreads from the same animal proved to differ little in this regard.

The results are best taken up in the consideration separately of Tallqvist and Willebrand (4), Dawson (5), Busch and v. Bergen (6), render a description of most of these unnecessary. Appended (6) render a description of most of these unnecessary. Appended are complete counts from the lymph of twenty-three dogs.

1. *Polymorphonuclear Neutrophiles.*—These were observed only in the presence of many red corpuscles, and then, with one exception, in such small quantity as the presence of blood in the lymph would quite account for. A little blood in the dog's lymph is practically the rule, as many previous observers have noted. Often its source is discovered in an anastomosis of the thoracic duct with small veins in the superior mediastinum, but occasionally it gains entrance further back in the lymph-system. The white cells introduced into the lymph in this way are few. That the polymorphonuclear neutrophiles in the normal lymph are due wholly to contamination was several times shown with some exactness by quantitative counts of the red and white cells of the lymph and of the blood, conducted with another end in view (Rous (7)). In these cases the ratio between polymorphonuclear neutrophiles and red cells in the lymph coincided with that found in the blood.

In one instance, as noted above (Dog H1), polymorphonuclear neutrophiles were found in an abundance that admixture of blood could not account for—11.5 per cent. of the 4,500 white cells per cubic millimeter of lymph. At autopsy of this animal its organs seemed normal macroscopically. The instance remains unexplained.

2. *Eosinophiles.*—These striking cells were almost always found

in the lymph, and often in considerable numbers,—12 per cent. of the total white cells in one instance. They average 2.6 per cent. Their independence of blood-admixture is shown by their relative abundance when compared with the polymorphonuclear neutrophiles. The finding has a special interest because of the well-known general relationship between the eosinophile and conditions of nutrition, and between the eosinophile and animal parasites. Accordingly steps were taken to find whether an eosinophilia of the lymph depends on these factors.

Heidenhain (8) first pointed out that the intestinal mucosa of the well-fed dog is thronged with eosinophiles. They are most abundant when the animal is on a mixed diet, and become fewer if meat is withdrawn from the food, or if there is overfeeding with it. Starvation causes a profound diminution in the cells. These observations have been confirmed on other animals. Opie (9) has recently shown that when food is withheld from the guinea-pig the number of eosinophiles in its blood, after a transient increase, falls off rapidly.

In the present instance the time that elapsed between the animal's last meal and the operation at which lymph-smears were obtained was purposely varied much. From the diet of half of the

TABLE I.

Series A.—Much Meat in Diet.

Dog.	Hours Since Last Meal.	Condition of Lymph.	Per Cent. of Eosinophiles in Lymph.	Parasites in Intestine.
Cl	5	Chylous.	11.6	No autopsy.
Hl	27	Thinly chylous.	2.4	One specimen of *Toxacara canis.*
Il	27	Slightly chylous.	7.8	*Dipylidium caninum* 0.5 gram.
Bl	27	Opalescent.	2.3	No autopsy.
Gl	27	Opalescent.	2.5	One specimen of *Toxacara canis.* *Dipylidium caninum*, 0.5 gram.
Ml	27	Opalescent.	6.9	*Tænia pisiformis* 1.7 grams.
Dl	30	Opalescent.	1.0	No autopsy.
Ll	52?[2]	Quite chylous.	2.9	*Dipylidium caninum* 0.5 gram.
Nl	52	Opalescent.	0.4	*Dipylidium caninum* 0.8 gram.
Kl	52	Nearly clear.	1.0	No parasites.
Jl	52	Nearly clear.	1.1	*Toxacara canis* 1.8 grams.

[2] Autopsy shows recent feeding. Stomach and small intestine contain much food.

Series B.—Meat Almost Wholly Excluded from Diet.

Dog.	Hours Since Last Meal.	Condition of Lymph.	Per Cent. of Eosinophiles in Lymph.	Parasites in Intestine.
Bt	2	Thinly chylous.	0.0	Two specimens of *Toxacara canis.*
C_2l	3	Thinly chylous.	2.8	*Dipylidium caninum* 0.2 gram.
Ft	3½	Chylous.	2.3	*Tænia pisiformis* 17 grams.
Rl	4	Chylous.	3.3	No parasites.
Ql	20	Clear.	1.3	*Dipylidium caninum* 1.0 gram.
Sl	27	Opalescent.	0.0	*Toxacara canis* 3.0 grams.
Yl	27	Opalescent.	0.0	*Tænia pisiformis* 1.9 grams.
Tl	27	Slightly opalescent.	1.2	*Tænia pisiformis* 16.0 grams.
Zl	27	Clear.	0.6	*Tænia pisiformis* 9.4 grams.
Xl	52	Slightly opalescent.	1.1	*Tænia pisiformis* 11.5 grams.
Pl	52	Clear.	1.6	No parasites.

dogs meat was almost wholly excluded, carbohydrates being substituted. The results are made plain by the following table.

These figures show that a mixed diet rich in meat considerably favors the occurrence of eosinophiles in the lymph, as compared with one of which meat forms a slight part. Furthermore, when all food is withheld the number of eosinophiles in the lymph becomes much fewer. Series A. demonstrates this especially well.

To determine the influence of animal-parasites a record was kept of those that autopsy of the dogs disclosed. Only such as were prominent macroscopically were noted. These were washed, fixed in formalin, and then weighed. The table gives the data resulting. No clear relationship between the presence of parasites and the number of eosinophiles of the lymph is to be seen.

One may well ask, whence come these eosinophiles? Are they the result of a local formation in lymph-glands and intestinal mucosa, or do they represent a migration, more or less indirect, from the blood-stream? Biedl and v. Decastello concluded that the eosinophiles formed in the lymph-glands of the dog must enter the blood directly, since only once did they come upon these cells in the lymph. But the present findings destroy the ground for that conclusion. Simon (10) has noted a few eosinophile myelocytes in the intestinal mucosa of the dog, though not sufficient to account for its content in polymorphonuclear eosinophiles. It is conceded that these, in the main, gather there from the blood. The fluctuation in the lymph's content of eosinophiles agrees remarkably with

that observed by others in the intestinal mucosa, and the fact suggests that this mucosa may be the proximal source of the eosinophiles of the lymph.

3. *Mast-cells.*—These were not seen in the lymph.

4. *Lymphocytes.*—In the study of the dog's blood several classes of these may be separated with reference to staining properties of nucleus and cytoplasm, relative proportion of the two, and size of the cell as a whole (Dawson). But in dealing with the lymph it was soon found a sufficient task to make one group of these cells as distinct from the large mononuclears. Botkin long ago called attention to the effect of pressure as determining the appearance of lymphocytes in spreads, and he held this responsible for much of the individual variation seen. The factor comes into special play in preparations made from the lymph, because this fluid lacks the "body" necessary to the production of uniform films. Frequently cells at the periphery of a lymph spread average twice the size of those at the center.

As a standard to separate large mononuclears from small the size of the polymorphonuclear neutrophile in the same specimen of lymph, or that failing, in the animal's blood, was adopted. A mononuclear element of which the nucleus was smaller than an entire polymorphonuclear neutrophile was classed as a lymphocyte, no matter what its amount of cytoplasm. Such elements constituted from 69.8 to 96.8 per cent. of the total white cells, with an average of 87.6 per cent.

5. *Large Mononuclear Leucocytes.*—Mononuclear elements with a round or oval nucleus larger than that just defined for the lymphocyte were classed as "large mononuclears." Occasional mitoses were observed in them. From 1.8 to 18 per cent. of such cells were found in the lymph, with an average of 5.1 per cent. The relation of these to the like elements in the blood is discussed further on.

6. *Transitional Leucocytes.*—The characteristic transitional cell of the dog's blood is slightly larger than the polymorphonuclear neutrophile. The nucleus is indented, often bilobed or deeply cleft into two or three irregular, blunt branches. With Wright's stain the cytoplasm is a distinct blue, usually of light tint, as compared

with the pink, more or less granular, cytoplasm of the polymorphonuclear neutrophile; and the nucleus is a medium dark blue in contrast with the dense, purplish blue of that of the latter cell. The most complex nucleus of a transitional is thick and clumsy as compared with that of a neutrophile, and the chromatin is much less compact. The cytoplasm occasionally shows a few neutrophile granules. All gradations between this cell and the typical large mononuclear exist, but, as has been brought out, it can always be sharply distinguished from the polymorphonuclear neutrophile.

In my preliminary observations on the lymph transitional leucocytes were but seldom noted, indeed much less frequently than polymorphonuclear neutrophiles. To test this finding, in the later counts all mononuclear elements with indented nucleus (even cells in evident process of division, and those with reniform nucleus) were put under the head of "transitionals." Yet despite this latitude the group remained a small one. In one case (Dog Pl) 1.6 per cent. of these cells were present; but altogether, out of 10,511 leucocytes counted, only forty-one were "transitionals." At least half of these forty-one were mononuclear elements in process of division. In the lymph of six of the twenty-three animals "transitionals" were not met with during an average count of five hundred cells.

This is in marked contrast to the frequency of transitional leucocytes in the dog's blood. For the purposes of such contrast special differential counts were made of smears taken at time of operation from the animals yielding the lymph. The counts of the authors already quoted could not be utilized, since in these the transitional and large mononuclear leucocytes are lumped under one head. In the special counts (which are appended in full) cells with a merely reniform nucleus are termed "large mononuclears," and only those with a nucleus more deeply indented are accepted as transitional leucocytes. Thus the class of transitional forms is made radically narrower for the blood than for the lymph. Yet in the blood these cells occur in the proportion of 1 to every 3.1 lymphocytes, whereas in the lymph there is only 1 to every 224 lymphocytes. The table shows this fact.

TABLE II.

Transitional Leucocytes and Lymphocytes.

	Lymph.			Blood.		
Dog.	Transitionals.	Lymphocytes.	Total Count.	Transitionals.	Lymphocytes.	Total Count.
Bl	4	452	526			
Cl	3	477	684	11	78	512
Dl	0	445	502			
Fl	1	469	500	10	47	315
Gl	0	504	550	25	16	311
Hl	0	405	545	21	45	317
Il	0	431	510	15	72	344
Jl	5	332	363	27	51	320
Kl	1	284	303	14	41	306
Ll	1	478	519	21	106	305
Ml	2	457	536	14	69	310
Nl	0	467	492	12	64	400
Pl	5	257	307	9	80	400
Ql	1	261	300			
Rl	1	442	509			
Sl	2	484	500	41	36	500
Tl	1	370	402	19	36	400
Xl	4	358	402	15	60	320
Yl	2	371	400	13	32	306
Zl	3	461	500			
Bt	3	314	335			
C_2l	0	461	526			
Ft	2	226	300			
	41	9,206	10,511	267	833	5,366

Plainly the transitional leucocytes of the blood cannot be furnished to it as such through the thoracic duct. True, it is possible to suppose that the production of transitional forms ceases immediately that the animal is operated upon; hence the scarcity of these elements in the lymph. But the other cells are not so affected, and we have no reason to believe that this occurs. The conclusion seems justified that the transitional leucocytes of the blood do not come to it as such in the lymph. They either develop in the blood from other lymph elements or are derived from another source.

Furthermore, if the cell-character of the lymph represent the general type of contribution made by all lymphoid tissue to the blood, then one is forced to conclude that lymphoid tissue either furnishes transitional leucocytes to the blood under the guise of some other element, or else has no hand in their production.

Are similar conclusions for the large mononuclear leucocyte justified? The counts show the existence of from 1.8 to 18 per

cent. of these cells, with an average of 5.2 per cent. in the lymph of the thoracic duct; and these are, of course, added as such to the blood. Nevertheless, a comparison of the ratios between the large mononuclear leucocytes and lymphocytes in the blood and in the lymph, respectively, gives results similar to, though not so striking as, those for the transitional leucocyte. For this comparison it is necessary to add to the number of "large mononuclears" of the lymph that of the few transitional leucocytes found, because cells with reniform nucleus found in this fluid were classed as "transitionals," whereas in the differential counts of the blood they were called "large mononuclears." Yet, despite this inflation of the number of "large mononuclears" of the lymph through the real transitional leucocytes introduced under this head, the ratio of large mononuclear leucocytes to lymphocytes in the lymph is 1 to 17, as compared with a 1 to 4.4 ratio in the blood.

TABLE III.

Large Mononuclear Leucocytes and Lymphocytes.

	Lymph.			Blood.		
Dog.	Large Mononuclears.	Lymphocytes.	Total Count.	Large Mononuclears.	Lymphocytes.	Total Count.
Cl	38	452	526			
Bl	85	477	684	19	78	512
Dl	45	445	502			
El	17	469	500	9	47	315
Gl	20	504	550	3	16	311
Hl	10	405	545	7	45	317
Il	16	431	510	6	72	344
Jl	18	332	363	16	51	320
Kl	6	284	303	15	41	306
Ll	14	478	519	15	106	305
Ml	20	457	536	9	69	310
Nl	19	467	492	20	64	400
Pl	27	257	307	18	80	400
Ql	13	261	300			
Rl	11	442	509			
Sl	14	484	500	13	36	500
Tl	12	370	402	7	36	400
Xl	37	358	402	20	60	320
Yl	19	371	400	13	32	306
Zl	24	461	500			
Bt	8	334	335			
Cl	17	461	526			
Ft	54	246	300			
	544	9,206	10,511	190	833	5,366

Despite these figures it is not necessary to suppose, as for the transitional leucocytes, that large mononuclear forms develop in the blood from other lymph elements, or else have a source outside the lymph. For, in the first place, the lymph certainly does add to the blood a considerable quantity of large mononuclear cells as such; and, second, a relative accumulation of large mononuclear cells might occur through their persistence in the blood for a longer period than the lymphocytes. Third, in the counts on which this discussion is based, the separation of the large from the small mononuclear elements was accomplished through their difference in size alone—a criterion which permits of large error from the subjective side. The separation of the transitional leucocytes rested on peculiarities of morphology and of tint which could not be overlooked.

7. *Unclassified.*—A considerable number of elements were put under this head. These were for the most part cells distorted

LYMPH COUNTS.

Dog.	Lymphocytes.	Large Mononuclears.	Transitionals.	Polymorphonuclear Neutrophiles.	Eosinophiles.	Unclassified.	Total Counted.	Remarks.
Bl	452	38	4	0	12	20	526	
Cl	477	85	3	4	79	34	684	
Dl	445	45	0	0	5	7	502	
El	469	17	1	1	0	12	500	
Gl	504	20	0	2	14	10	550	
Hl	405	10	0	63(!)	13	54	545	Only slight blood admixture.
Il	431	16	0	7	39	17	510	
Jl	332	18	5	3	4	3	363	
Kl	284	6	1	0	3	9	303	
Ll	478	14	1	1	15	10	519	
Ml	457	20	2	12	37	8	536	Considerable blood admixture.
Nl	467	19	0	4	2	0	492	
Pl	257	27	5	8	5	5	307	
Ql	261	13	1	4	4	17	300	
Rl	442	11	1	6	17	32	509	
Sl	484	14	2	0	0	0	500	
Tl	370	12	1	0	5	14	402	
Xl	358	37	4	0	3	0	402	
Yl	371	19	2	3	0	5	400	
Zl	461	24	3	3	0	9	500	
Bt	314	8	3	1	0	9	335	
C_2l	461	17	0	1	14	33	526	
Ft	226	54	2	3	7	8	300	
	9,206	544	41	126	278	316	10,511	
	87.6%	5.2%	0.39%	1.2%	2.6%	3.0%		

while the smears were being made, owing to the lymph's lack of "body." A few typical "basket cells" were observed.

Résumé.—Eosinophile cells are a frequent constituent of the lymph of the normal dog. Sometimes they are quite abundant: in one case noted they formed 12 per cent. of the total white cells and they averaged 2.6 per cent. When food is withheld from the animal their number becomes much fewer. The kind of food on which the dog has subsisted also exerts an influence, a mixed diet largely of meat favoring eosinophiles, as compared with one mostly of carbohydrates. The finding parallels so nearly that of Heidenhain in his study of the eosinophiles of the intestinal mucosa as to suggest this mucosa as the proximal source of the lymph's content of these cells. A systematic record of the animal parasites in the

BLOOD COUNTS.

Dog.	Polymorphonuclear Neutrophiles.	Eosinophiles.	Lymphocytes.	Large Mononuclears.	Transitionals.	Unclassified.	Total.	Remarks.
C[3]	350	44	78	19	11	10	512	
E[3]	216	22	47	9	10	11	315	Two normoblasts seen during count.
G[3]	248	15	16	3	25	4	311	
H[3]	205	32	45	7	21	7	317	
I[3]	207	39	72	6	15	5	344	Two mast cells and normoblasts seen during count.
J[3]	203	19	51	16	27	4	320	Three normoblasts seen during count.
K[4]	216	16	41	15	14	4	306	
L[4]	141	15	106	15	21	7	305	Two mast cells and nine normoblasts seen during count.
M[4]	195	19	69	9	14	4	310	One normoblast seen during count.
N[4]	271	21	64	20	12	12	400	
P[4]	274	10	80	18	9	9	400	
S[4]	360	38	36	13	41	12	500	
T[4]	289	48	36	7	19	1	400	
X[4]	202	14	60	20	15	9	320	
Y[4]	233	11	32	13	13	4	306	

[3] Smears made just after incision.

[4] Smears made just before operation.

dogs used did not bring out any definite relation between these and the eosinophiles of the lymph.

Mast-cells are not a constituent of the lymph of the normal dog. Polymorphonuclear neutrophiles are only present as a result of blood admixture. Lymphocytes, by which are meant non-granular cells with a round or oval nucleus smaller than an entire polymorphonuclear neutrophile from the same animal, form an average of 87.6 per cent. of the cells.

Typical transitional cells are rare in the lymph. Evidently those of the blood must either develop in it from other lymph-elements or must come from another source than the lymph, and thus from another source than adenoid tissue in general.

Large mononuclear cells—non-granular mononuclear elements larger than the lymphocyte as above defined—average 5.2 per cent. of the lymph's leucocytes.

Owing to the lymph's lack of "body" smears made from it show many distorted cells. Thus the class of "unclassified" elements is rendered comparatively large.

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OBSTRUCTIVE HYDROCEPHALUS FOLLOWING CEREBROSPINAL MENINGITIS, WITH INTRAVENTRICULAR INJECTION OF ANTIMENINGITIS SERUM (FLEXNER).[1]

By HARVEY CUSHING and FRANK J. SLADEN,

Baltimore.

Plate XXXI.

Despite the ultimate fatality and the failure to secure a post-mortem examination in the case of this unfortunate patient, the interest, from a therapeutic standpoint, of finding, long after the primary infection, the meningococcus still viable in the cerebral ventricles in association with an obstructure hydrocephalus, whereas the spinal fluid retained no trace of organisms, makes it seem desirable to place upon record even this single experience with the intraventricular injection of serum.

History.—A baby six months old was brought by its mother to the children's dispensary of the Johns Hopkins Hospital on the 21st of March, 1908, with the statement that the child had been wasting for four month and its head enlarging.

There was nothing in the parent's story as then related to Dr. J. H. M. Knox, the physician in charge, to indicate that the condition was other than a simple so-called essential hydrocephalus, but owing to the unusual degree of tension of the head and the infant's apparently critical condition Dr. Knox was insistent that some immediate steps be taken to even temporarily relieve the pressure, and the child was brought by him to the surgical operating room where, as will be related, lumbar and ventricular punctures were performed.

On *physical examination* the patient (Fig. 1) proved to be a frail, weak, semi-comatose baby possessing a greatly and somewhat asymmetrically enlarged head with widely separated sutures and fontanelles. The cranial enlargement was the more apparent when contrasted with the emaciated trunk and limbs. The skin over the small body hung in folds; the ribs were prominent; the abdomen was scaphoid. There was a macular rash over the chest and abdomen. Rectal temperature 99.6°; pulse 110; weight 3,900 grms.

There was a convergent strabismus; the pupils were dilated but reacted to light. Conjunctivæ were clear. Ears were negative. No herpes. No erupted teeth; nursing reflex active.

[1] Received for publication May 15, 1908.

The head was large, measuring 47 cms. in its greatest circumference. The fontanelles were widely opened and tense; in the center of the anterior fontanelle lay an island of bone measuring about 5 cms. in diameter (Fig. 2). All of the palpable sutures were separated. The tense scalp was covered with greatly dilated and tortuous vessels. There was a network of dilated venules in the eyelids. The general appearance of the head was that of infantile idiopathic hydrocephalus.

The head was markedly retracted; neck very stiff and resistent to passive movement; no arching of spine. There was a coarse tremor and marked spasticity of all four extremities. The arms were held flexed at elbows in "driving position"; the legs were occasionally (more usually the left) held flexed at the knee and ankle, but a slight stimulus would cause them to straighten out with accompanying dorsal flexion of the great toes.

The deep reflexes were all exaggerated; Babinsky's toe phenomenon was bilaterally positive. Kernig's sign was not definite; the limbs could be easily straightened by using a little force.

Thoracic and abdominal viscera were practically negative.

Lumbar and Ventricular Puncture No. 1.—Only a small amount of clear fluid was obtained from the lumbar meninges—not enough to diminish appreciably the intracranial tension; consequently a left ventricular puncture was performed at the outer angle of the open fontanelle, 120 c.c. of clear fluid being removed. Cultures were taken from each specimen, and smears were immediately examined, the lumbar fluid showing no organisms, whereas the ventricular fluid contained an abundance of Gram-negative, intracellular diplococci. The cultures from the lumbar fluid remained sterile, whereas those from the ventricular fluid gave a diffuse heavy growth, which proved to be a pure culture of the *Diplococcus intracellularis meningitidis* (Weichselbaum).

Not until the smears were examined did we for a moment suspect that there had been a previous meningeal infection—far from anticipating the actual presence of living organisms. In consequence of this disclosure the child was admitted to Prof. Barker's service, where, in the light of our acquired information, the following somewhat more detailed history (indicative of an acute illness, probably meningitis) was extracted from the parents.

Family History.—Unimportant.

Past History.—This, the fourth child, was born at full time after an easy labor. The head was not large at birth. For the first three months the child was breast-fed; since then, under a physician's direction, it had been put on a variety of changeable diets.

Present Illness.—Four months ago the child, then a seemingly normal baby of only eight weeks, awoke early one morning evidently suffering from some derangement of acute onset. This was followed an hour later by a severe convulsion which produced unconsciousness. On the following day there occurred four or five other convulsions, and again three weeks later still another of the same character, though there had been none in the interval. For several days and nights after the onset the child was feverish and screamed constantly. The eyes are said to have become crossed, the neck stiff and the head drawn back. There was no continuous eruption; no herpes.

The child had been treated during these four months by various physicians for pneumonia, muscular rheumatism, indigestion, intestinal rickets, etc.

In addition to the notes on the child's physical condition given above, a blood examination was made after entrance showing white blood corpuscles 17,000, polymorphonuclears predominating; red blood corpuscles of variable size; none nucleated.

March 22.—Lumbar puncture No. 2 was pertormed, only 2 c.c. of fluid being obtained: no evident increase of tension. Fifteen c.c. of antimeningitis serum (Flexner) were injected, causing evident discomfort. Microscopically the sediment of the centrifugalized fluid showed no organisms and hardly any cellular elements—only a few leucocytes were found. The cultures remained sterile, confirming the observation made on the fluid removed the day before at *Lumbar Puncture No. 1.*

March 24.—Child seems somewhat better, taking nourishment well. Fontanelles remain less tense than when first seen, that is, before first ventricular puncture. Child lies quietly on its side with head retracted and eyes widely open. Does not appear to see, though pupils react to light.

March 28.—The tension has gradually increased until the fontanelles have become fully as tight as on admission; circumference of head 46.5 cm. *Ventricular Puncture No. 2.* No anæsthesia; needle introduced through outer angle of fontanelle into left ventricle, presumably through second frontal convolution. Clear fluid, unstained with blood, rose in capillary tube to height of 95 cm., though child was quiet without crying or straining. Tube was lowered and 150 c.c. of clear, limpid fluid containing a few flucculi were slowly withdrawn; 15 c.c. of antimeningitis serum were then injected. Child's condition was unaffected one way or another; no vomiting followed the puncture as on the first occasion.

The fluid contained intra- and extracellular diplococci in considerable numbers. There was a diffuse growth on glycerine agar from two loops of the centrifugalized sediment.

The rectal temperature rose, after the puncture, to 101.2°, but the general condition on the following day seemed somewhat inproved.

March 30.—After atropine instillation a satisfactory view of the eye-grounds was obtained for the first time on this day, Dr. Bordley finding a choked disc of at least 3 D. on the right and 2 D. on the left, with extremely large and tortuous veins near the discs.

March 31.—Ventricular Puncture No. 3. Though the fontanelles are somewhat less tense than before and the child evidently gaining, the puncture was repeated; ventricular tension was not measured; 100 c.c. of fluid were withdrawn from the right ventricle and 15 c.c. of antimeningitis serum introduced.

The head measured 46 cm. before tapping, and 45.5 cm. afterwards, showing its ready collapsibility.

The fluid obtained was clear, though yellowish in tint, evidently stained by serum. A centrifugalized specimen showed diplococci in undiminished numbers, though possibly there were more intracellular and fewer extracellular organisms than before. Smears from two loops of the sediment again gave a diffuse growth on glycerine agar.

For two hours after the tapping clear fluid leaked from the puncture wound; the bandage and pillow were stained yellow and it was thought that much of the serum might have been lost. The head became so greatly collapsed that the thin shells of bone overlapped at the sutures.

April 1.—The general condition seems much improved; cheeks and body in general seem to be filling out. Actual gain in weight, however, is not great, possibly owing to loss of fluid. Head gradually refilling.

April 3.—Child irritable; crying much; head again tense, measuring 46.5 cm. *Ventricular Puncture No. 4.* Fluid rose in capillary tube only 50 cm.; 60 c.c. removed and 15 c.c. of antimeningitis serum introduced through needle.

The fluid was clear with no trace of color to suggest serum, much of which may have leaked out after the injection of March 31st. Smears showed diplococci both in and out of cells though greatly diminished in number. On culture two loops of the centrifugalized sediment gave isolated colonies instead of a diffuse growth as before.

April 5.—The rectal temperature again showed a transient rise to 101.8° after the puncture of two days ago. General condition, however, seems to be much improved. Weight 3,970 grm.

April 6.—Weight 4,150 grm. Child doing splendidly; sleeps well, takes nourishment greedily. Head less tense than on any previous occasion 48 hours after puncture. Condition thought to be so favorable that the contemplated retapping of the ventricle was postponed.

April 8.—During the early morning hours, from some unaccountable cause, the patient became cyanotic and developed Cheyne-Stokes respiration. When seen at 7:30 A. M. the baby was in a state of collapse; in a cold sweat; eyes sunken; pulse weak and hardly perceptible; respiration gasping.

The fontanelles had become tense during the night, and as a last resort a *ventricular puncture* (No. 5) was made, in the hope of relieving the pressure symptoms; 140 c.c. of clear, slightly yellowish fluid were removed. The child's condition was not appreciably changed by the procedure, and in spite of stimulation the pulse and respiration gradually grew weaker and the child died six hours later. The rectal temperature did not rise above 99°.

Cultures taken in the routine fashion (two loops of centrifugalized sediment on glycerine agar) from the fluid at this last tapping gave only eight to ten colonies. The smears showed a great diminution in the number of organisms. Some polymorphonuclear leucocytes contained indistinctly staining Gram-negative diplococci; there were very few extracellular organisms to be seen.

Comment.—It has been our impression that the fatalities in many of the cases of cerebrospinal meningitis that we have seen during the past few years have occurred through the medium of cerebral pressure rather than as the result of an overwhelming intoxication or terminal infection. Although not fully dwelt upon in the autopsy notes of each case, in most instances nevertheless the existence of a ventricular dilatation has been recorded. Unfortunately it has not been an invariable custom to harden the brains in situ, and consequently the full extent of the pyo-hydrops ventriculorum may in some cases have escaped notice.

As early as 1898 attempts were made in the Johns Hopkins Hos-

pital to treat some of the more desperate cases of cerebrospinal meningitis by surgical measures. At that time a few patients[3] were subjected to a lumbar laminectomy and a permanent drain was established after washing out the subarachnoid space as thoroughly as possible by retrograde irrigation. One of these patients recovered from the meningeal infection to die of pyo-nephrosis some two months later; another after a period of apparent improvement succumbed to the infection, and the meningeal spaces and basal cisternæ were found post-mortem so occluded with a fibrinoplastic deposit leading to an internal hydrocephalus that it was impossible to conceive of a lumbar drain helping the condition in any way.

In view of these and similar experiences a median suboccipital drainage with opening of the exposed posterior cistern has more recently been adopted and has seemed to promise more than did these lumbar operations; nevertheless, even in these cases, an obstructive hydrocephalus may develop. For this reason during the past two years a number of patients with evident ventricular obstruction have been subjected from time to time to ventricular aspiration. Some few of them have for the time being been markedly improved, although without exception they were all practically in extremis at the time of the operation. The brief history of one of these cases may be cited in illustration.

The patient, a sailor, was brought to the Johns Hopkins Hospital from a North German Lloyd steamer on January 19, 1907. During the voyage another sailor had died from meningitis. The patient was exceedingly ill with the characteristic symptoms of cerebrospinal fever. From the time of his admission to February 7 repeated lumbar punctures—twelve in all—were performed. After the earlier punctures there was considerable improvement; after the later ones none, and finally no further fluid could be obtained. A double choked disc appeared, complete nerve deafness, strabismus, delirium, etc. A cranial operation with puncture of the lateral ventricle at Keen's point of election was performed by Dr. Sowers. There was an almost immediate and a very marked improvement in the patient's condition; he regained consciousness, the choked disc and headache subsided, the temperature became normal, and there were no further untoward symptoms until February 15, when there was a sudden and unexpected exodus.

At the autopsy it was evident that the infection had largely subsided, leaving,

[3] Some of these cases were recorded by Dr. Osler in his Cavendish Lecture. *West London Medical Journal*, 1899, iv, 145.

however, a thick membrane which effectually closed the outlets for ventricular fluid in the neighborhood of the fourth ventricle. The lateral ventricles which were considerably dilated (Fig. 3) contained sero-purulent fluid. The lining surface, particularly of the left ventricle, showed the characteristic granular ependymal inflammation.

Before the days of serum treatment the experiences with lumbar puncture, as in this instance, usually proved symptomatically beneficial up to a certain period of the illness, at which time the punctures would often fail even temporarily to give relief, supposedly owing to the small amount of fluid, if any, which could be obtained. Since the serum injections following lumbar puncture have become a routine with us in the treatment of this disease, its course has almost always been less severe and it seems unquestionable that there has been less of a fibrino-plastic exudate than in the cases which were treated in earlier days by a lumbar puncture alone. This would make it seem probable that under the serum treatment there is a lessened likelihood of the particular complication we are considering.

However, even among our later cases there have been a certain number of fatalities, evidently due to a ventricular block, and in all instances in which an autopsy has been permitted internal hydrocephalus has invariably been found. Such a stasis of ventricular fluid in the closed skull of an adult leads naturally to critical pressure symptoms, and in the reduced physical condition of these patients the complication is often fatal. It is notable too that these signs of cerebral compression—the stupor, increased headache, the choked disc and so on—may supervene with considerable abruptness in the course of an infection which seemed to be progressing favorably, and not uncommonly late in the disease. In an infant, on the other hand, as in this patient the history of whose illness has been detailed, the pressure effects may be warded off through the possibility of cranial enlargement owing to separation of the sutures.

In case, therefore, the acute pressure symptoms can be alleviated, either by operative measures or, in an infant, by the method of relief through cranial distension, it is quite conceivable that the dilated ventricles may continue to hold organisms which remain a menace for a considerable period of time. For this to occur there

presumably must be a discontinuity between the ventricular cavities and the subarachnoid spaces of the brain and spinal cord. So far as clinical evidence can be relied upon, it certainly appears to be conclusively shown in this patient that such a discontinuity existed and that the organisms had completely died out of the spinal meninges and had retained their viability within the ventricles alone.

Late complications, associated oftentimes with a low grade of hydrocephalus and accompanied by irritative symptoms due to cortical changes and to cortico-meningeal adhesions, have in the past not uncommonly been seen after recovery from meningitis—complications which it is to be hoped will be greatly lessened under a more widespread use of serum treatment. It is but natural that they should be seen more frequently after recovery from the disease in childhood than in adult life, and it is well known that a low grade of persistent ventricular distension is not incompatable with subsequent and even unusual mental vigor, provided there has occurred a reopening of the outlets for the escape of fluid.

We have seen a number of cases in which, for some months after the primary infection, symptoms of ventricular distension recurred with a more or less definite periodicity, the pressure symptoms being absent in the interval. In some patients indeed, even after an interval of freedom, they have recurred and persisted. Some of these cases have been subjected to operation and one of them in particular we wish to mention:

The child had been desperately ill for six months during the epidemic of three years ago in New York, and his recovery was long despaired of. Blind and deaf, totally paralyzed for many weeks, there was a gradual slow restoration to fairly normal health. The child, however, began to have frequent convulsive seizures, many of them Jacksonian in character, and also began to fail mentally. An ophthalmoscopic examination showed a low grade of choked disc. A bilateral exposure of the hemispheres disclosed the presence of innumerable fine adhesions between the pia-arachnoid and overlying dura. These adhesions were separated over both hemispheres, the ventricle was aspirated, and at the same moment a lumbar puncture was performed. On lowering the capillary tube used to measure the tension of the fluid in the lumbar region, fluid was withdrawn in large amounts, evidently escaping from the ventricles by this lumbar route, for the somewhat protruding brain subsided markedly. It was the operator's impression that with an open skull the atmospheric pressure against the hemisphere was sufficient to allow a rechanneling of the membranous thickenings over the fourth ventricle, which enabled the pent up fluid to again find

its way from the ventricles into the subarachnoid spaces and thus to escape by its normal channels. The child completely regained its normal health.

This history is cited merely to emphasize the importance of these intracranial complications even in cases of recovery from cerebrospinal meningitis and the possibility of their at least occasional betterment through surgical measures.

Noteworthy in the case the subject of this report are the following facts—namely, the obstructive hydrocephalus; the long-standing ventricular infection with the meningococcus in the presence of sterile fluid in the spinal meninges; and finally the marked diminution in number of the organisms in the ventricles as the result of serum injection even in a case of such long standing.

The case suggests that it may be advisable in other instances of ventricular obstruction in the acute stages of the disease to perform, even in the adult, ventricular punctures and, if organisms are present, to administer serum, with proper precautions, directly into the ventricle.[8] It is possible that in this way the mechanical factors at work in producing the compression may be relieved for a time sufficiently long to tide over the period of plastic inflammation about the brain stem and thus to enable the channels of exit for the fluid from the ventricles again to become reopened.

[8] Unusual care, of course, particularly in the closed skull of the adult, must be observed not only in the ventricular aspiration but more particularly in the reintroduction of serum; for it would be even more hazardous in the case of the ventricle than in the lumbar subarachnoid space to introduce more fluid than has been removed. Flexner has wisely cautioned against this.

In the infant the ventricle may be safely entered through the outer angle of the open fontanelle at a point about 2.5 cm. from the median line. In the adult, on the other hand, cranial penetration is necessary, and in all of our cases, except in the instance cited in this report, Kocher's point of election has been chosen (Fig. 3). There is no especial difficulty in the procedure. A small patch of scalp is shaved and a short one-inch linear incision made about 3.5 cm. from the mid-longitudinal line and about 5 cm. anterior to the Sulcus centralis; the bone is exposed and penetrated by a Doyen perforator followed by a burr, which leaves a cup-shaped fossa and gives sufficient exposure of the dura to assure the operator that there is no large underlying cortical vessel. The hollow exploratory needle, which should have a blunted point with openings upon the side, is then gently inserted into the second frontal convolution perpendicular to its surface, and at a depth of from 4 to 5 cm. readily finds the ventricle, particularly if it is distended. (Cf. chapter in Keen's "System of Surgery," 1908, vol. iii, p. 117.)

EXPLANATION OF PLATE XXXI.

FIG. 1. Photograph showing general appearance of the child. Note enlargement of head and dilatation of superficial vessels, the retraction of the neck with no arching of the spine, the hypertonicity of the muscles and tremor of the extremities, the dorsal flexion of the great toes.

FIG. 2. Photograph of top of head showing separation of sutures and widely opened anterior fontanelle containing isolated island of bone. Note dilatations of vessels.

FIG. 3. Showing degree of ventricular dilatation in fatal adult case of cerebrospinal meningitis. Section of brain after hardening in situ by carotid injection of formalin. Arrow shows direction of ventricular aspiration at point of election.

FIG. 1.

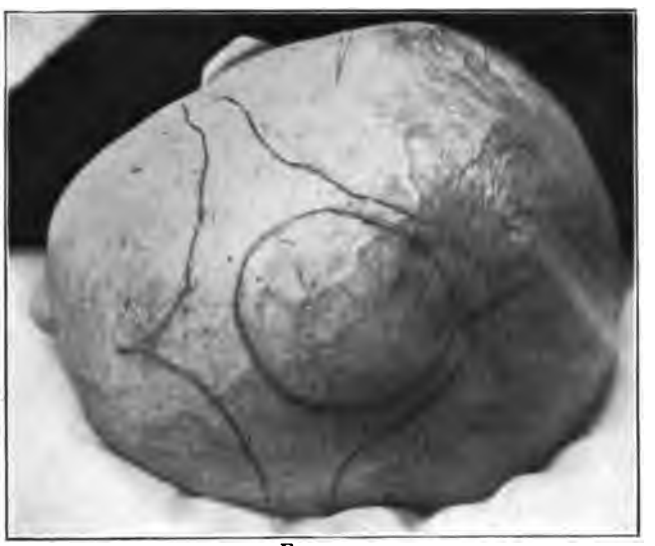

FIG. 2.

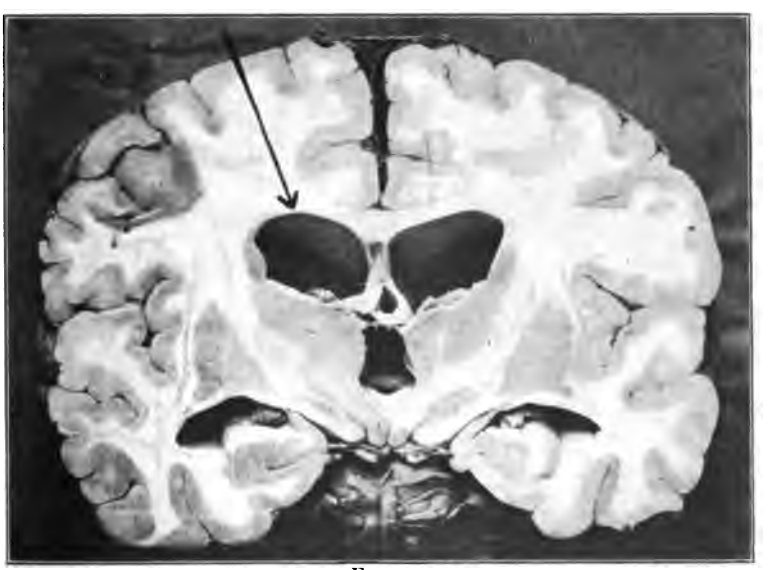

FIG. 3.

ON GLYCOTHIONIC ACID.

By P. A. LEVENE and W. A. JACOBS.

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Of all the substances belonging to the class of glycothionic acids only one has been the subject of detailed chemical analysis—namely the one discovered by Mörner,[1] first carefully studied by Schmiedeberg and later by Neuberg and Orgler[2] and by S. Fraenkel.[3] As yet it is uncertain whether or not all the substances of this group are identical or different in their chemical structure.

In the present communication a brief report of the analysis of the acid obtained from tendo-mucin and of that obtained from the spleen is given.

It was found possible to obtain a barium salt of the acid possessing a constant composition, which could be best expressed by the formula $C_{14}H_{19}NO_{14}SBa + H_2O$.

	Calculated for this Formula.	Found.
C	27.30	27.29
H	3.43	3.64
N	2.29	2.58
S	5.05	4.85
Ba	22.34	21.90

The analysis of this barium salt has made it probable that its molecule is composed of sulphuric acid, acetic acid, glycuronic acid and aminoglycuronic acid. This conclusion was arrived at on the following grounds.

On hydrolysis with hydrobromic acid with simultaneous oxidation with bromine there was obtained a silver salt with the properties of saccharic acid. The silver calculated for the formula $(HCOH)_4(COOAg)_2$ and the amount found were:

	Calculated.	Found.
Ag	50.94	50.44

[1] *Skand. Arch.*, 1889, i, 210.
[2] *Zeitsch. f. physiol. Chem.*, 1903, xxxvii, 407.
[3] *Liebig's Ann.*, 1907, cccli, 344.

This can be regarded as an oxidation product of glycuronic acid.

Besides, there was obtained a barium salt which had the composition of a mixture of equal parts of aminoglycuronic acid and of glycuronic acid.

	This mixture Requires	Found.
C	27.58	27.27
H	3.64	4.50
N	2.68	2.47
Ba	26.29	25.77

On hydrolysis of the substance in autoclave at 175° C., a silver salt of a substance was obtained which possessed the composition of that of the monoacetylglycuronic acid.

	This body Requires	Found.
C	21.43	21.52
H	2.45	2.40

On hydrolyis with 25 per cent. sulphuric acid in autoclave a silver salt of acetic acid was obtained. That the substance contains no hexose one is justified to conclude from the fact that no levulinic acid was obtained on heating the substance with 25 per cent. sulphuric acid and on the ground that a phenylosazon is obtained from the products of hydrolysis of the substance with great difficulty. This osazon had a melting point = 155° C.

There is also no ground to believe that the substance contains in its molecule a pentose, since on distillation with hydrochloric acid a quantity of furfurol is obtained which does not correspond to that required by the presence of a pentose.

The substance is levorotatory. The barium salt of the substance obtained from the spleen differed in its composition from that of the tendo-mucin.

	Tendo-mucin.	Spleen.
C	27.29	29.74
H	3.64	3.82
N	2.58	3.64
S	4.85	....
Ba	21.90	13.89

THE HUMAN SPLEEN AS AN HÆMATOPLASTIC ORGAN, AS EXEMPLIFIED IN A CASE OF SPLENOMEGALY WITH SCLEROSIS OF THE BONE-MARROW.[1]

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Plates XXXII and XXXIII.

This paper has been written for the following reasons:

1. To present a case of splenomegaly with very unusual pathological findings.

2. To show the similarity which may exist between bone-marrow changes and splenic transformation, and the relation of the one to the other.

3. To consider certain types of splenomegaly as being compensatory processes dependent upon and due to bone-marrow disease.

History of the Case.—The patient, Samuel K., an oiler, aged 58 years, was brought into the Pennsylvania Hospital, August 5, 1907, in the ambulance during the service of Dr. J. C. Wilson, whom I wish to thank for the following notes.

He complained of malaise, weakness, dyspnœa, shortness of breath, insomnia, loss of appetite, nausea, constipation and œdema of lower extremities. His father died of "debility"; his mother, as result of an accident. Three brothers and two sisters are dead, the causes unknown. Three brothers and three sisters are living and well. There has been no chronic illness in family. The patient was born in County Dongon, Ireland. He lived there until he was thirteen years old; he came to the United States when fourteen, worked on a farm for three years and then was employed by Traction Company as driver and conductor for twenty-three years. He was employed as "workman on road" up to 1902. Since then he has done odd jobs. During the last two years he has been employed as an oiler in an engine room. He has always been a healthy individual. He had measles, mumps and whooping cough when a child. Patient states that as far as he knows he has never been sick before the present illness (with the exception of diseases of childhood). There is no history of malaria, syphilis or gonorrhœa. He drinks a fair amount of tea and coffee. He occasionally drinks

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whiskey and gin as well as considerable beer—15 to 20 glasses a day. He has taken no liquor for past three years.

Patient thinks that his illness began about the first of this June, 1907, with a swelling of the lower extremities, dyspnœa and shortness of breath which gradually increased; he was unable to sleep or lie down with any degree of comfort. Appetite was poor; he was nauseated and vomited once or twice; bowels were regular; there was no cough nor pains in chest. There was occasionally slight vertigo. There was no abdominal pain, distension or tenderness. Patient worked until the middle of June when he was compelled to stop as his condition was becoming worse. He went home and remained there but did not go to bed. Symptoms gradually became more marked; his feet and legs became more œdematous, dyspnœa and shortness of breath increasing with slight precordial pain; appetite became poorer. He began to notice that the abdomen and scrotum were becoming swollen. He was unable of late to lie down on account of dyspnœa. Sleep was very poor. On admission to the hospital temperature was 99° F.; pulse, 108; and respiration, 36.

Physical examination shows a fairly well developed though emaciated man, past middle age. He sits up in bed and is unable to lie down. He breathes with difficulty and is swollen and anemic looking. Abdomen and extremities are moderately swollen and œdematous and pit on pressure. Pupils are equal and react to light and accommodation. Conjunctiva is clear though anæmic looking. The tongue is moderately coated, moist and not tremulous; the breath is offensive. There are some pulsations of vessels of neck. There are no enlarged glands; no rigidity. Brownish pigmentation is present at the root of the neck. Chest is fairly well developed and symmetrical; expansion is poor. At the base of both lungs there are some crepitant and mucous rales, with slight impairment to percussion; breath sounds are somewhat suppressed and distant, but otherwise the lungs are clear. There is normal vesicular breathing with no impairment of resonance; vocal fremitus is fair.

The area of cardiac dullness is slightly enlarged, extending to the upper border of the third rib and 3 cm. to the right of the mid line; the heart beat is circumscribed and moderately forcible. There is also seen and felt just at the edge of costal margin 1.5 cm. to left of the mid line, an impulse. Heart sounds are heard fairly well at all the cardiac areas; they are somewhat forcible but not clear and distinct, and seem to lack muscular tone. At apex there is heard, at times, a soft systoic murmur transmitted to the base. The pulse is moderately rapid, fairly soft and compressible and at times slightly irregular. The arteries are slightly sclerotic. The abdomen is quite distended and tense and pits on pressure in the lower quadrants. On percussion there is dullness in the flanks, and the lower quadrants are slightly tympanitic below costal angle; there are definite succussion splash and movable dullness. There is a large mass in left upper and lower quadrants, apparently projecting from below the costal margin. It is quite hard, apparently solid and smooth, and tender to palpation. The mass extends from the costal margin into the pelvis, approximately measuring 23 cm. and from the median line to left flank, 23 cm. It does not move with respiration and moves only slightly on change of position. Coils of intestine can be felt floating over the mass. Liver reaches from the fourth rib in mid-clavicular line to the costal margin and is tender on palpation. There is no pulsation. The

mass in the left quadrant is apparently the spleen or else is fused with it. Pelvis and scrotum are somewhat swollen and œdematous. There is moderate œdema of the lower extremities.

On admission the hæmoglobin was 85 per cent. (Dare). The blood count was as follows:

Leucocytes, 11,550 per cu. mm.

Polymorphonuclear leucocytes	71.6 per cent.
Large mononuclear leucocytes	13.2 per cent.
Small mononuclear leucocytes	8.4 per cent.
Transitional leucocytes	6.0 per cent.
Eosinophile leucocytes	0.4 per cent.
Basophile leucocytes	0.4 per cent.

Urine is reddish yellow, acid, sp. gr. 1023; there is a large amount of albumin; no sugar; hyaline and granular casts, a few leucocytes, epithelial cells, and mucous cell detritus are found.

Patient improved for a time but gradually became worse and died of asthenia September 10, 1907. After the first day the temperature never advanced above normal point.

The blood was examined from time to time while the patient was in the hospital but apart from the fact that there were evidences of a gradual decrease in the hæmoglobin, a slight decrease in the number of red blood corpuscles and a moderate increase in the large mononuclears, the blood picture showed nothing unusual. The lowest percentage of hæmoglobin was 50 per cent.; the minimum number of red blood cells 3,220,000; of white blood cells 8,950. The differential counts were suggestive of nothing. At no time in the course of the disease were nucleated red blood cells found.

An autopsy was performed seven and one half hours after death. The report is as follows:

A. 1013 D. The body is that of a well built, rather poorly nourished adult male, measuring 155 cm. in length. Rigor mortis is present to a slight degree. Bluish red discoloration of dependent parts. Pupils are equal, clear and widely dilated. No lymph glands palpable. External genitalia apparently normal. The abdomen is protuberant.

Abdominal cavity contains approximately 500 c.c. of a clear straw-colored fluid. The bowels are in a collapsed condition and the omental fat is scanty. The parietal and visceral peritoneal surfaces are smooth and glistening with well-injected vessels. The mesenteric glands are enlarged. The appendix is directed downward, inward and upward in the right iliac fossa: it measures 5 cm. in length, is patent throughout and apparently normal. The spleen is seen to extend 5 cm. below the costal margin.

Each pleural cavity contains approximately 1,000 c.c. of a yellow-brown, clear fluid. There are a few adhesions over the right pleura; the left lung is bound to the chest wall by numerous adhesions. The pericardial cavity contains about 50 c.c. of a clear straw-colored fluid. The visceral pericardium is covered, in numerous areas, by large white plaques which are firmly adherent to the underlying tissue.

Heart.—Weight, 450 grm. The right ventricular wall measures 8 mm. in

thickness, the left ventricular wall 3.25 cm. in thickness. The heart's flesh is of a pale red color, very firm in consistency and there is evidence of an increase in the interstitial connective tissue. The coronary arteries are patent throughout and free from sclerosis. Tricuspid, aortic and pulmonic valves are negative. The anterior leaflet of the mitral valve is somewhat hard in consistency and apparently slightly thickened. Five millimeters above one of the aortic cusps is a calcareous area measuring 1 cm. in diameter.

Lungs.—The right lung is voluminous, crepitant in some portions and boggy in other areas. It is of a purple color. No nodules are felt. On section a grey fluid exudes from a cut surface which is of a gray-black color dotted with small areas of a dark red. A few white fibrous plaques are found in the right pulmonary artery. The left lung is somewhat smaller than the right one and is tougher to the feel. The external surface is purplish grey in color. On section the upper lobe resembles the right lung. The lower lobe is, however, entirely collapsed and is of a dark red-brown color; on pressure it exudes a slight amount of frothy red fluid. There are a few sclerotic patches in the left pulmonary artery.

Mesenteric glands are greatly enlarged throughout and on section present areas of caseation and calcification.

Spleen.—22 x 15 x 8.5 cm.; weight, 1,470 grm. Its external surface is covered with small and large fibrous tags. The organ is very firm and regular to the feel. Small whitish plaques, varying in size from that of a millet seed to a split pea, are noted over its entire surface. On cut section the capsule is seen to be somewhat thickened and the organ, as a whole, of a dark red color. The trabeculæ are very distinct. The Malpighian bodies are barely visible. Scattered throughout and indiscriminately arranged are small, rather soft areas of a light red color, spherical in form, varying in size from a pin point to that of a large bean. These soft nodules stand out prominently as light red areas in contradistinction to the surrounding tissue which is of a much darker red. The above described portions do not appear to be encapsulated, though they are definitely spherical.

Kidneys.—The right kidney measures 8 x 6.5 x 4 cm. The capsule strips quite readily though it is slightly adherent in areas. A small retention cyst is noted. On section the cut surface is gray-red in color. The cortex measures 8.5 mm. in thickness. The glomeruli are rather prominent; the pyramids and striæ very distinct. The pelvic fat is normal in amount.

The left kidney measures 10 x 4.5 x 3 cm. It is somewhat lobulated and rather soft in consistency. The capsule strips with difficulty. On section the cut surface is gray-red in color. There is an evidence of confluency of cortex with the pyramids, no distinct line of demarcation being seen. The glomeruli are quite distinct, as are also the striæ and pyramids. There is a small cortical retention cyst.

Liver.—23 x 12 x 9 cm.; weight, 2,130 gms. It is very hard to the feel. The external surface is free from adhesions and is of a pink-gray color. The edges are slightly rounded. On section the cut surface is reddish yellow in color, studded with small patches of yellow-red, especially noted toward the center of the liver lobules. There is a marked engorgment of the capillaries. The portal connective tissue is apparently slightly increased in amount. The bile ducts are patent; no stones are found. Vessels are apparently normal.

Gall bladder contains approximately 50 c.c. of a viscid brown fluid.

Adrenals are apparently normal.

Urinary bladder is apparently normal.

Testicles are somewhat œdematous but there are no signs of orchitis. There is a small cystic tumor of the right epididymis.

Prostate and seminal vesicles are apparently normal.

Aorta is apparently normal.

Gastric mucosa shows congestion and is covered with thick mucus; otherwise it appears normal.

Pancreas is apparently normal.

The intestinal mucosa especially in the neighborhood of the big gut shows a marked hyperplasia and is covered by thick mucus such as is seen in the stomach. In the neighborhood of the jejunum there is marked vascular injection.

Bone marrow removed from the middle portion of the right femur shows a very firm dark brown-red tissue.

Anatomical Diagnosis.—Acute œdema of lungs. Dilatation and hypertrophy of right and left ventricles. Chronic myocarditis. Chronic splenic tumor. Atelectasis of lower lobe of right lung. Chronic parenchymatous nephritis. Cloudy swelling of liver and kidneys. Tuberculosis of mesenteric lymph glands. Sclerosis of bone marrow. Fibrinous pleurisy.

MICROSCOPICAL EXAMINATION.

Heart.—Muscle fibers are of normal size but are quite granular and contain a fair number of vacuoles. There is some increase in the interfibrillar connective tissue.

Lung.—Pleura is somewhat thickened. Many of alveoli are filled with a serous-like material. Capillaries are engorged; no large giant cells are seen.

Liver.—Capsule shows moderate thickening. The liver cells are swollen and granular and show a well advanced grade of vacuolization. Some areas show so high a degree of granulation that the liver cords have completely lost their contour and appear piled upon one another. Extending from the center of many of the lobules and in some portions almost to the periphery, are areas of necrosis with complete disappearance of the liver parenchyma; they have been filled with red blood corpuscles, vast numbers of polymorphonuclear leucocytes and an occasional round cell. The connective tissue around the bile ducts shows slight thickening with round cell infiltration. The periportal connective tissue is somewhat increased. The inter- and intralobular connective tissue does not appear to be increased in amount. The blood vessels and capillaries are filled with red blood cells. Their walls are normal. No multinucleated or large vesicular cells are found in any sections.

Kidneys.—Attached to the cortex are bands of connective tissue which show a moderate degree of round cell infiltration. At the juncture of the cortex with these fibrous bands, are large clusters of round cells. Many of the glomeruli appear normal in all respects, others are atrophied and still others have undergone a complete hyaline change. The epithelium of the tubules is swollen and granular and many of the lumina are almost occluded by the swollen and desquamated cells. There is a slight increase in the intertubular connective tissue. The blood-vessel walls are slightly thickened. The capillaries and larger vessels are filled with red blood cells. No multinuclear or large vesicular nucleated cells are found.

Pancreas.—The gland is apparently normal.

Mesenteric Lymph Glands.—Capsule shows moderate thickening with extensive round cell infiltration. The sinuses are in areas entirely obliterated by the proliferative and desquamative endothelial cells of the lymph spaces. Many of the lymphoid follicles have been entirely replaced by a necrotic tissue surrounded and infiltrated by large epithelioid, round cells and giant cells of the bipolar and mural type. Scattered within the tissue are polymorphonuclear leucocytes, especially seen around the areas of necrosis. Many of the germinal follicles seem to be the starting point for the tuberculous process. No bone marrow giant cells or vesicular nucleated cells (except the epithelioid cells) are seen.

Bone Marrow.—There is an enormous hyperplasia of the cellular and connective tissue elements. The types of cells seen are of all the varieties seen in the bone marrow under normal conditions (Fig. 3). Thus the types seen are, neutrophilic and eosinophilic myelocytes, polynuclear leucocytes, giant cells, large and small mononuclear cells, normocytes, normoblasts and megoblasts, together with large numbers of fibroblasts of the connective tissue. The cells as a whole lie embedded in a very much thickened and well-formed connective tissue membrane containing well-developed blood vessels and capillaries (Mallory's connective tissue stain). The neutrophilic myelocytes are for the most part arranged in clusters and are occasionally seen, bunched together in spaces lined with endothelium. The cells described as myelocytes are the fairly large mononuclear cells which appear to contain neutrophilic and eosinophilic granules; no true basophilic myelocytes are seen. Scattered indiscriminately throughout the tissue are large giant cells which appear in two forms (though their nuclei which often contains a nucleolus and their nuclear fibrillar network together with their hyaline appearing protoplasm, are the same) : one type of cell possesses numerous nuclei, arranged generally in the center, and is composed of large vesicular nuclei and nucleoli with a well-defined network and surrounded by a hyaline appearing protoplasm. To this type is applied the term polykaryocyte; the other type or megokaryocyte possesses but one singular lobulated nuclear cast, possessing a nucleolus, well-defined nuclear membrane and surrounded by a large amount of hyaline appearing protoplasm. Throughout the section are noted enormous numbers of very large cells with vesicular nucleus about which is a small, apparently non-granular, rim of protoplasm, which has a tendency toward taking the alkaline stain. These cells contain often a well-developed nucleolus. They have a tendency toward cluster arrangement, and some definite masses of these cells are seen in spaces lined by endothelial cells. Arranged among erythrocytes and also scattered indiscriminately among the other cells are fairly large numbers of normoblasts, some of whose nuclei are in the process of extrusion. An occasional megoblast with dividing nucleus may be seen. The polynuclear cells are scarce as compared with those of normal bone marrow, though here and there are noted some. They have no tendency toward arrangement in clusters. There are definite large masses of small mononuclear cells or lymphocytes seen in different portions of the sections. An occasional polynuclear eosinophile with no definite position is seen. Coursing everywhere are enormous numbers of fibroblasts and larger and more developed connective tissue cells. The blood vessels are large and well developed with thickened walls. The capillaries are markedly increased also. There are no organisms seen in sections. A

noticeable feature is the presence of very large numbers of small mononuclear cells and fibroblasts with a relative decrease in the normal functionatory marrow cells.

Spleen.—Capsule is somewhat thickened, is infiltrated with round cells and has attached to it small and large strands of connective tissue. The trabeculæ and blood vessels show a slight hyperplasia; there is a marked increase in the reticulum. Many of the Malpighian bodies appear intact though the majority of them show some atrophy, many of them having disappeared. The splenic pulp is markedly hypertrophied and is found to contain large clusters of well-formed and well-preserved red blood corpuscles. Scattered here and there among the pulp cells are seen very large cells with vesicular nuclei, some cells containing but a rim of a homogeneous protoplasm, while in others the protoplasm is more extensive and apparently contains what are perhaps neutrophilic granules. There is a marked tendency on the part of these cells to cluster together and lie within spaces lined by epithelial cells. An occasional multinucleated giant cell of the bone marrow type is seen, many normoblasts are visible. There is an extraordinarily small amount of pigment.

Splenic Nodes.—Surrounded by a tissue which microscopically resembles the above are areas from 1 to 2 cm. in diameter, which greatly differ in some respects from the surrounding tissue. These areas (Fig. 1) are seen to be made up almost entirely of large cells, containing a vesicular nucleus and but a rim of protoplasm. They for the most part are seen in small spaces, some of which appear to be lined with endothelial cells. Bone marrow giant cells, both of the multinuclear and polynuclear forms, are seen in abundance (Fig. 2). All these cells are supported by a connective tissue meshwork, which contains also large numbers of nucleated red cells and erythrocytes arranged in no particular fashion. No pigment is seen.

Microscopic Diagnosis.—Œdema and congestion of lungs. Fibrinous pleurisy. Chronic interstitial myocarditis. Fatty metamorphosis of heart and liver. Chronic splenic tumor, with bone marrow cell hyperplasia. Cloudy swelling of heart, liver and kidneys. Chronic diffuse nephritis. Primary tuberculosis of mesenteric lymph nodes. Sclerosis of bone marrow.

As the term splenomegaly is now applied it has especial reference to a splenic hypertrophy which has extended over a period of months, perhaps years. Some writers include under the word splenomegaly only those conditions in which there is an idiopathic enlargement of the spleen, accompanied by an anæmia or perhaps an anæmia with a cirrhotic liver. Others apply the term to a condition in which there is an enlarged spleen with known or unknown etiology. The causes of many of the splenomegalies is still so uncertain that to formulate any definite classification from an etiological standpoint is wholly impossible. It is not the purpose of this report to discuss all of the various conditions which may give rise to an enlarged spleen. The main interest arises as to the

etiology of these cases grouped under the term "splenic anæmia." At the present time their origin is attributed to some intoxication, chronic in form, which principally affects the spleen, though sclerosis of the splenic and portal vessels and thrombosis of the splenic sinuses have been given as possible causative agents. That a diseased bone marrow might be the contributing cause in some of the cases has not been suggested, and it is important, therefore, to present an example in which the bone marrow is as extensively affected as the spleen.

This appears to be the condition in the case just described. What toxin, if any, caused the chronic inflammation of the bone-marrow in this case is not known. It should be remembered, too, that from the histological examination the bone-marrow presents a more intense and, perhaps, more advanced sclerosis than does the spleen, and that the greater part of the bone-marrow elements, as seen in the sections, are not the true blood-forming cells of the body, but are principally lymphoid cells and fibroblasts. Associated with this intense sclerosis of the bone-marrow are the definite foci composed of true bone-marrow elements in the spleen.

It is needless to add that a differential diagnosis from the other splenomegalies is not necessary. From a review of the literature it has been seen that the case described above does not resemble the types heretofore reported.

The pathological changes which take place in the various forms of splenomegaly, particularly of the primary types, are not uniform and the relative bearing of the bone-marrow upon cases of splenomegaly has been but briefly commented upon. It is, therefore, only by careful examination of individual cases and by experimentation upon animals that we may hope to arrive, some day, at conclusions as regards the physiologic and pathologic properties of the splenic hypertrophies.

In consideration of all cases of enlarged spleen, especially when the etiology is entirely unknown, we must of necessity turn to the so-called hæmopoëtic organs in which group are included, besides the spleen, the bone-marrow, lymph glands, liver and hæmolymph glands. That a decided relationship exists between the first three has been proved by all who have spent much time in study of the

subject. The liver too undoubtedly plays an important rôle, while Warthin's (1) work on the "Anatomy and Physiology of the Hæmolymph Glands," together with W. B. Drummond's (2) monograph on the same subject, have brought these structures into an important relationship with the other tissues considered as exciting some special action on the blood's function or destruction. Bizzozero and Neuman, long ago, demonstrated the fact that the bone-marrow was the seat of formation of the red blood cells. Soon after Ehrlich not only verified this statement, but also demonstrated that the bone-marrow was the site of white cell formation. Investigators of later date have found these facts to be true, and we are now in the position to give the most important functions of the bone-marrow. The function of the lymph gland cannot be asserted as definitely, but the fact remains that the lymph glands cannot be excluded from those organs described as being hæmopoëtic. They are possibly the place of origin of the lymphocytes of the circulating blood and from chemical and experimental observations it appears that they have power to assume, at some time, perhaps a compensatory function. The liver in embryonal life aids in the blood's formation, while as age advances it gradually loses that power and helps in the carrying off of waste matter, the result of disintegration of red cells. The functions of the hæmolymph glands are still in dispute; thus Drummond (2) claims that "there is no sufficient evidence that the hæmolymph glands play any part in the formation of red blood cells, but that on the other hand they appear to perform a very active part in the destruction of red blood cells and in the liberation of pigment." On the other hand, Warthin (1) says that "the close relations between spleen, lymph glands and bone-marrow is shown by the power of the hæmolymph glands to take on a structure of either spleen or marrow and to compensate for these organs when their function is abridged by disease." And finally the spleen has perhaps caused more discussion than any of the other organs. Differences in the cellular contents and structural characters in various animals has proven a barrier for decisive experimental work. Thus in lower vertebrates and in some of the mammalia, as the mouse, hedgehog and rabbit, nucleated red cells are normally present, giving evidence of a formation of red blood

cells in the spleen. Ehrlich (3), on the other hand, states that in the human spleen nucleated red blood cells are not to be found normally. Other investigators do not substantiate this phase of Ehrlich's work and claim that under normal conditions normoblasts are often found in the human splenic pulp. It is for this reason extremely difficult in animal experiments to determine the part taken by the spleen in forming erythrocytes. It is generally believed that though the spleen may be one of the sources of origin of red blood cells, its main duty is the absorption of the material due to disintegration of red and white cells and to preserve a portion of it, at least, for the organism.

The main point of interest which concerns us is the significance that may be attached to a pathological change in the spleen in which we see definite bone-marrow elements, including giant cells arranged in isolated areas through the splenic pulp. The presence of giant cells, resembling in all respects those of the bone-marrow existing in the human spleen, has been mentioned and but slightly commented upon by Dock and Warthin (4), Rolleston (5) and Simonds (6). Dock and Warthin interpret them as being emboli, while Rolleston and Simonds offer no hypothesis. On the other hand, numerous investigators, such as Dominici, Opie, Jarotsky, Bunting and others have commented upon their presence in the hedgehog, the rabbit and the guinea pig, under normal as well as under pathological conditions. Under all normal conditions bone-marrow giant cells are absent from the human spleen. It is only during an acute or chronic intoxication that they appear in the human spleen. I have not been able to find a note on their presence in the acute infections of human beings, though in one case which I have studied the spleen from a patient dying with acute gonorrhœal endocarditis, showed definite bone-marrow cells, including the true myeloplaxes and the large granuleless mononuclears arranged in small clumps. Dock and Warthin's report on the pathology of two cases of " splenic anæmia " is interesting since in one of their cases giant cells resembling those of the bone-marrow were found in great numbers in the lung capillaries, while a smaller number were found in the liver capillaries and blood spaces of the spleen and hæmolymph glands. In the other case there were found large

numbers of these cells in the lungs, a smaller number in the spleen and a fair number in the hæmolymph glands. In both cases there was a decided decrease of giant cells in the bone marrow. As stated above, the writers considered these cells emboli.

The question now arises whether there are splenomegalies in which the bone-marrow's function has become exhausted by the action of some circulating toxin and in which the spleen exerts itself in a compensatory manner by forming new blood elements for the organism.

Osler, Sippey (7) and Simonds (6) have thoroughly reviewed the literature of splenomegaly and hence the citation of cases will be dwelt upon only so far as it may have a definite bearing on the subject under discussion. The cases of so-called "splenic anæmia" or splenic anæmia with cirrhosis of the liver (Banti's disease) form the greater part of the cases of obscure origin. In these the pathology has been quite well outlined by Rolleston (5), who says that the main pathologic changes in splenic anæmia are: (1) Fibrosis of the organ, (2) atrophy of the lymphoid elements and (3) fibrosis of the Malpighian bodies. In addition there is usually no evidence of exaggerated hæmolysis such as excessive pigmentation of the spleen. He notes also that there is usually a red marrow transformation, though the yellow marrow may persist.

The pathological changes seen in the present case are entirely different from those which have been described previously. Macroscopically distinct small, rather soft areas, of a light red color, spherical in form, varying in size from a pin head to that of a large bean, were quite striking. Simonds (6) describes an enlarged spleen as containing "numerous round, oval or branched reddish brown areas about two millimeters in diameter." In this case the areas, containing a large amount of pigment, give an intense iron reaction. The difference is obvious, since the areas described in my case are free from pigment and are seen to be made up of collections of what appear to be definite bone-marrow cells. There was great similarity between sections of the bone-marrow and sections through these areas in the spleen. For a comparative study of the bone-marrow and the cells in the nodules the most useful stains were Duval's modification of Leishman and Wright stains,

polychrome methylene blue, hæmatoxylin and eosin, Mallory's connective tissue stain, Van Giesen's stains and Ehrlich's triacid stain. With none of these stains, however, have definite neutrophilic granules been made out in the cells, either in the spleen or bone-marrow. This is perhaps due to the fixation which unfortunately was entirely in Zenker's fluid. Some of the cells of the bone-marrow presented, what appeared to be, neutrophilic granules. There were cells in the spleen which had the same appearance, but I cannot state positively whether they were or were not myelocytes. It can be definitely said, however, that the giant cells, the megakaryocytes, were present in both tissues and that the large granuleless mononuclear cells found normally in the bone-marrow alone were present in great abundance in these nodules in the spleen.

The question now arises whether this enlargement of the spleen should be considered as an heteroplastic growth or as an hyperplastic. We meet with three hypotheses: (1) That the condition is due to a disease of the spleen *per se,* (2) that the bone-marrow foci found in the spleen are emboli and the enlargement due to an intense congestion, (3) that the enlargement depends upon the spleen assuming a dormant hæmopoëtic function with hyperplasia of the cells of the spleen pulp.

The exact determination as to whether the condition was primary in the spleen is impossible. From the examination of the bone-marrow it seems more probable that the sclerosis there is of an older form and of a greater intensity than it is in the splenic pulp. The theory that these masses of bone-marrow tissue arise from bone-marrow cell emboli cannot hastily be disposed of, for the two cases reported by Dock and Warthin, referred to above, which showed definite bone-marrow cells in the capillaries of the liver, spleen, hæmolymph glands and lungs, together with Bunting's (10) report of the finding of definite bone-marrow in the dorsal portion of the aorta, would perhaps suggest the possibility of such an emigration of cells. Bunting, however, states that one is not justified in deciding whether they were formed by a further metaplasia of connective tissue cells or by a metaplasia of emigrated cells from the blood stream, capable of differentiation into the various types of marrow cells. In my case it does not seem probable that the

bone-marrow elements have been brought to the spleen by the blood stream, for if such were the case it would seem reasonable to suppose that these same cells should be found in the lungs and liver, as in the two cases of Dock and Warthin. On the other hand, possibly such a process cannot be entirely ruled out for the clusters of marrow cells, some of which are found in blood spaces, suggest at least the idea that they may be emboli. However, from a careful study of the sections as regards the position of the bone-marrow cells and the condition of the lymph nodes, liver and lungs, I do not feel justified in stating that they are brought directly to the spleen from the bone-marrow.

Finally we come to the third proposition as to whether the enlargement may be due to the development within the spleen of blood-forming elements. This hypothesis really rests upon two conditions, namely: (1) that the bone-marrow cells are fœtal remains of cells which have been lying latent and through a stimulant, perhaps exciting the bone-marrow to activity and finally to uselessness, have increased in proportion and have taken on their original fœtal blood-forming function; (2) that the spleen has produced bone-marrow cells from the blood or lymph spaces. Before arriving at any conclusion whatsoever as regards the logic of either of these two hypotheses, it is necessary to consider the subject, as best we can, from an experimental standpoint, for it is only by this means that we may throw some light on the subject. Indeed from the sections of organs removed at autopsy no consecutive changes can be noted.

It is an accepted fact that the human and mammalian fœtal spleens contain a varying number of bone-marrow cells such as the giant and myelocytic cell together with large numbers of nucleated red blood corpuscles. As the human spleen becomes further developed the bone-marrow and normoblastic cells gradually disappear while in the spleens of many of the mammals the blood foci cells persist through life though in greatly decreased numbers.

Experiments of Pugliese (11) on hedgehogs have shown that after repeated blood lettings the giant cells increase markedly in the spleen, and that after partial splenectomy the giant cells, in the intact splenic tissue, show a decided increase; that with total extir-

pation of the spleen the bone-marrow becomes filled with giant cells and that after repeated venesections the giant cells increase in the spleen but remain unchanged in bone-marrow, whereas in the liver and lymph glands there are no traces of them. The experimental anæmias produced by Bunting (12) in rabbits throw much light on the compensatory power of the spleen. Thus after sclerosis of the bone-marrow had been produced the spleen showed a marked dilatation of the peripheral venous sinuses which were crowded with marrow cells, and especially numerous were the megakaryocytes. Myer and Heincke (13) have shown similar changes in the spleen in a case of pernicious anæmia. Bunting (12) comments upon the fact that bone-marrow, following injection of hæmolytic toxins, becomes sclerotic and there may be an almost entire replacement of the hæmopoëtic elements by the newly formed connective tissue, while hæmorrhages cause only the hyperplastic marrow of a secondary anæmia. Clinically it has been noticed that an enlargement of the spleen often causes an improvement in the general condition of the bloods.

Dominice (14) has subjected rabbits to repeated bleedings and has found practically the same histological changes in the spleen as has Bunting. He has observed, however, that in the course of experimental infections with the typhoid bacillus there takes place what may be called "a partial myeloid change." By this term Dominice means that all the elements of the bone-marrow are not present. Thus there are noted large normoblasts and basophilic and neutrophilic myelocytes, but the eosinophilic myelocytes and giant cells are very rare or entirely absent. Jarotsky's (15) experiments with white mice infected with the hog cholera bacillus further substantiates the findings of Bunting, Pugliese, Dominice and others. Jarotsky produced in twenty-four hours an enormous increase of giant cells and myelocytes in the spleen.

Opie (19) has observed the occurrence of eosinophilic myelocytes and bone-marrow giant cells, in the spleens of guinea-pigs four hours after inoculation with Bacillus pyocyaneus, Bacillus anthracis, and Bacillus mucosus capsulatus—he believes that these elements are derived from the bone-marrow and are not formed in the spleen.

With the exception of some of Bunting's work on the experi-

mental anæmias, investigations of the subject have been limited to the acute infections. It is indeed fortunate that cases have been recorded in which similar splenic changes have taken place in the course of what appears in the present state of our knowledge to be a condition of chronic intoxication or infection, namely pernicious anæmia. Careful histological study of seventeen cases of pernicious anæmia by Gulland and Goodall (16) shows that in eight of the seventeen cases cells like those of the bone-marrow were found in the liver, while in four cases myelocytes were found in the spleen, and in one case there were typical large bone-marrow giant cells in the spleen.

Dominice and Jarotsky have written at length upon the possible mode of origin of the bone-marrow cells in the spleen. Their findings, though interesting, are not at all convincing. The theories of transportation of bone-marrow cell to the various organs can be substantially upheld by anatomical evidence in the leukæmias. That the phenomenon takes place, however, in all cases, where cells resembling those of the bone-marrow are found in the various organs, cannot be supported. On the other hand, experimental and anatomical evidence go to substantiate, if not prove, the theory that the spleen under certain conditions of chronic inflammation of the bone-marrow may take up the hæmopoëtic function. What the toxin is cannot be proven in many cases; but whatever the chemical changes may be, the toxin must have some deleterious effect upon the bone-marrow, either prohibiting the growth of cells or so stimulating them as to finally exhaust their power to produce blood cells. It is but natural to suppose that the spleen lying dormant, as it were, in the body should at times be called upon to perform or at least aid in performing the activities of the marrow.

CONCLUSIONS.

1. The nodules found in the spleen are islands of active hæmatoplastic tissue.

2. The bone-marrow, at least in the case which has been described, has been the primary focus of disease; some toxin has probably produced a chronic inflammatory change.

3. The bone-marrow, owing to its enormous sclerosis, has lost

its hæmopoëtic powers totally or to a marked degree and the spleen has reverted to its fœtal power to form blood.

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Explanation of Plates XXXII and XXXIII.

Fig. 1. Section from localized nodule in spleen (low magnification).
Fig. 2. Section from nodule in spleen (high magnification).
Fig. 3. Section from bone-marrow.

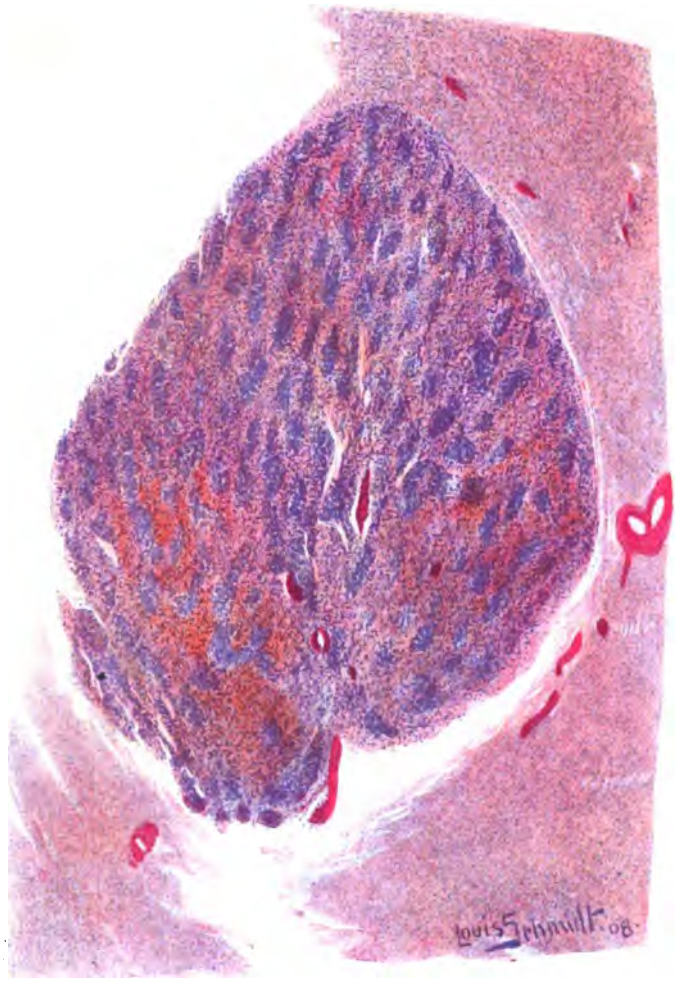


FIG. 1.

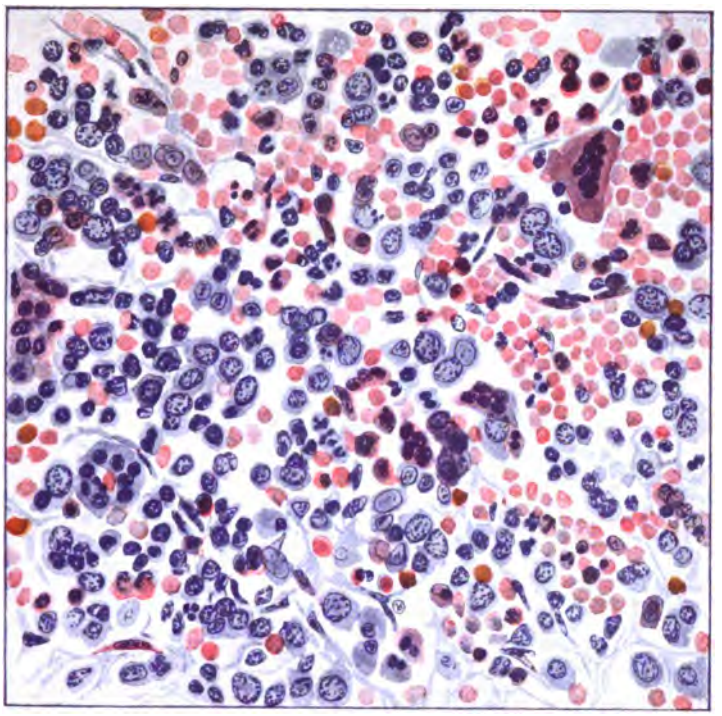


FIG. 2.

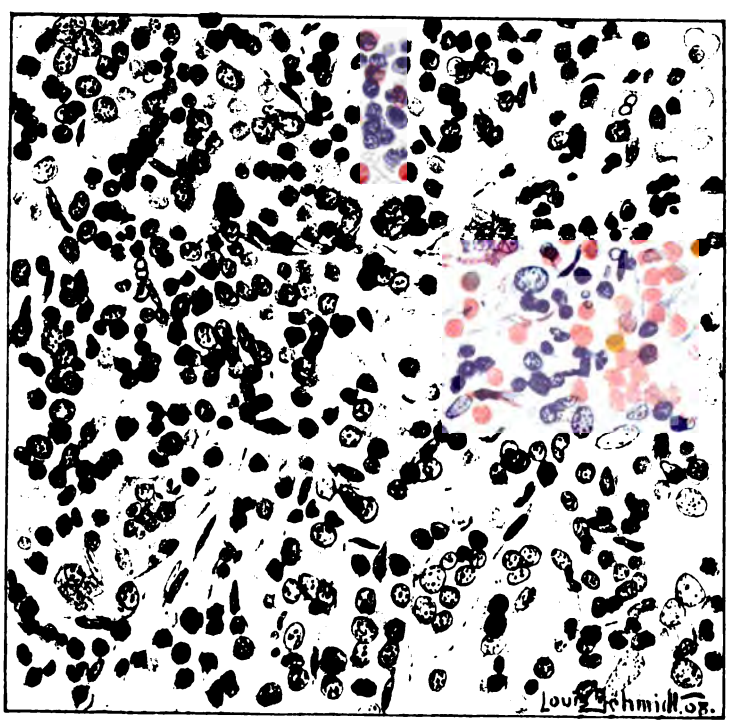


FIG. 3.

THE RESULTS OF THE APPLICATION OF SPECIAL HISTOLOGICAL METHODS TO THE STUDY OF TUMORS.[1]

By F. B. MALLORY.

PLATES XXXIV–XLVII.

The report which I have the honor of presenting to-night before this society on "The Results of the Application of Special Histological Methods to the Study of Tumors"[2] is a continuation of work reported in 1905 under the title of "A Contribution to the Classification of Tumors."[3] The results thus far obtained are of unequal value. Some groups of tumors have yielded much of interest, other groups little or nothing. I shall, therefore, limit what I have to say to those tumors which have proved of special histological interest. I shall first state briefly what has been found and then by means of lantern slides made from photomicrographs and drawings demonstrate visually as far as possible the same results.

The histological methods most used have been two, staining with phosphotungstic acid hematoxylin and with the anilin blue connective tissue stain after fixation in Zenker's fluid. In certain cases other fixatives and other stains have been employed. The value of these two staining methods is that they demonstrate clearly and sharply the fibrils which characterize certain cells and thereby render them easily recognizable. In the course of this work it has been found imperative to employ perfectly fresh tissue whenever possible, that is, tissue obtained at the operating table, cut into slices one to three millimeters thick, and placed immediately in the fixing solution. Zenker's fluid was found to be the best fixative for nearly all purposes. Formaldehyde proved

[1] Received for publication June 5, 1908.

[2] The Middleton Goldsmith Lecture of the New York Pathological Society delivered at the New York Academy of Medicine on April 4, 1908.

[3] *Journal of Medical Research*, 1905, xiii, 113.

of little value. Most of the tumors on which this report is based were placed in fixatives within two seconds to ten minutes after removal from the body.

The aim of this study of tumors has been to render simpler and more exact if possible than is at present the case, the histological classification of tumors. The histological classification is the one used in distinguishing and grouping the cells of normal tissues. It depends on the differentiation of cells and of the secretions and intercellular substances produced by them, and is the only practical method available for tumors unless the cause or causes of them should be discovered; then an etiological classification might be possible.

A histological classification of tumors demands a careful study of normal tissue elements in order to discover as far as possible how one kind of cell differs from another so that the same points of distinction can be applied to tumors. The obvious reason for this study of the normal tissue elements is the well known fact that tumor cells tend to differentiate more or less perfectly like the normal cells to which they are related. This differentiation of cells in their cytoplasm and intercellular substances is seen especially in the slower growing and older parts of a tumor where, perhaps, the supply of nutrition is not so abundant as to favor rapid proliferation. The method of study indicated here is not new. The only credit that can be claimed in this work, is, perhaps, a more rigid application of well-known principles and the use of some newer histological methods.

Much still remains to be done even in this narrow field of the histological classification of tumors, but it is a subject which is fundamental and useful in many other lines of tumor work. It calls for intelligent coöperation on the part of clinicians and pathologists. The latter are helpless without material with which to work, and that material must be obtained perfectly fresh, not brought to the laboratory in dry gauze or cotton twenty-four hours after removal, or placed whole in a minimum of weak alcohol, or of formaldehyde, or even of carbolized water or common salt solution. The clinician with his control of material and his knowledge of the histories of cases has in his power the

opportunity to prove of great aid to the advancement of this branch of medical research. The pathologist for his part must realize the possibilities of more exact diagnosis and replace as far as he can such indefinite terms as spindle cell sarcoma, round cell sarcoma, and perithelial angiosarcoma, for example, with more exact terms, for each of the tumors thus characterized may originate from several different kinds of cells.

The ordinary *connective tissue cell or fibroblast* is a flat cell with a flat oval nucleus and delicate cytoplasm spreading out in one plane. It is definitely characterized by the production of two kinds of fibrils; fibroglia fibrils which run along the surface of the cytoplasm in the direction of the long axis of the cell, are straight or gently curved in their course, and pass on apparently from one cell to the next; and collagen fibrils which lie alongside of the cell but not attached to it and are fine and wavy in appearance. These two kinds of fibrils can be readily differentiated from each other by several different staining methods. The source of elastic fibrils is not yet determined, but it is certain that they are not regularly produced by every fibroblast and, therefore, they do not concern us here.

The cells of tumors arising from cells of this type tend to differentiate like the normal cells. They are flat cells with oval nuclei and produce both kinds of fibrils. The collagen fibrils are always the more abundant and the more easily stained and recognized. They surround in equal amount all cells growing at the same rate of speed. In fibromata the fibroglia fibrils like the cells themselves are few in number. The tumors are composed chiefly of a mass of collagen fibrils. In the more slowly growing fibrosarcomata (Plate XXXIV, Figs. 1, 2 and 3) the fibroglia fibrils are more prominent, but in those in which the cell proliferation is rapid the fibrils are very delicate and are seen with difficulty except when viewed on end. In fibrosarcomata containing large multinucleated cells (Plate XXXV, Figs. 1, 2, 3 and 4) these large cells often produce coarse fibroglia fibrils, but in giant cell sarcomata, while the spindle-shaped cells produce both kinds of fibrils, the giant cells produce neither. No difference was found in the tissue ele-

ments of those fibrosarcomata in which the cells grow in broad bands and of those in which narrow bands of cells twist and twine in every direction.

In keloids the cells produce both kinds of fibrils and the fibroglia fibrils were particularly prominent in one case of rapid recurrence.

There seems to be little reason for putting the myxoma and myxosarcoma in a class by themselves. The cells produce both kinds of fibrils in abundance as can be readily demonstrated by staining a section of a well-preserved umbilical cord at term. The only essential difference between a fibroblast and a myxoma cell is that in the latter the collagen fibrils are more or less separated from each other in places by a varying amount of fluid containing mucin. These tumors should be regarded at the most as a variety of the fibroma and the fibrosarcoma.

The *smooth muscle cell* is a long spindle-shaped cell with a more or less rod-shaped nucleus and dense cytoplasm which stains deeply with acid dyes, especially eosin. Sometimes the cytoplasm of the cell is abundant and it tapers very gradually beyond the ends of the nucleus; at other times it is less in amount and contracts abruptly, and then continues for three or four times the length of the nucleus in each direction as a small round rod tapering to a point at the end. The outer surface or cuticle of the smooth muscle cell is striated longitudinally. These striations, termed myoglia fibrils by Heidenhain,[4] run close together at the ends of the cells so as to form in those cells in which the cytoplasm is slight in amount and rod-like, what seem to be coarse fibrils.

The myoglia fibrils are not so regular and well defined as either the neuroglia or the fibroglia fibrils, and they undergo post-mortem changes much more quickly. As far as can be determined from stained sections and from teased preparations they are limited to the cell to which they belong. This point is not easy to determine absolutely because smooth muscle cells overlap each other with great regularity and are closely cemented together as well as being surrounded with a varying number of collagen fibrils.

In leiomyomata (Plate XXXVI, Figs. 1 and 2) the cells tend to

[4] *Ergebnisse der Anatomie und Entwickelungsgeschichte,* 1898, viii, 3.

undergo the same differentiation as the normal smooth muscle cells, and the development of myoglia fibrils is usually equally marked. As a consequence these tumors are sharply characterized and easily recognized even with the ordinary stains because of the shape of the cells and the density of the cytoplasm with its property of staining deeply with acid dyes. The cells vary much in diameter and in length in different tumors, but that causes no difficulty in the diagnosis. In the rapidly growing leiomyomata, however, the differentiation of the cells may be slight or even in the most rapidly growing parts of the tumors entirely wanting; then a tumor may be easily called a spindle cell sarcoma and regarded as a fibrosarcoma, or sometimes on account of the shape of the cells be classified as a round cell sarcoma. Three cases of rapidly growing and clinically malignant leiomyomata of the uterus will illustrate the points I wish to emphasize.

In the first a large tumor, dense in places but soft and friable elsewhere, involved the fundus of the uterus, and smaller nodules were present in the broad ligament. Complete removal was not possible and death followed in less than three years, apparently as the result of continued growth of the tumor. In this case the new growth was composed of well-differentiated smooth muscle cells with few collagen fibrils binding them together. Mitotic figures were numerous, showing that proliferation was active. The diagnosis of a rapidly growing, probably clinically malignant, leiomyoma was easy to make.

In the second case a tumor the size of a cocoanut in the posterior wall of the uterus was connected at its base with a mass the size of an orange projecting into the lumen of the uterus. The tissue of both masses was for the most part grey, soft, and friable like a sarcoma. Microscopically the tumor showed extensive invasion of the wall of the uterus. It was composed in large part of rather large spindle-shaped cells (Plate XXXVII, Fig. 2) showing the fibrils characteristic of smooth muscle cells. Mitoses were numerous. The rest of the tissue was made up of large round and multinucleated cells (Plate XXXVII, Fig. 3) without fibrils and showing even more mitotic figures. The two kinds of cells, spindle-shaped and round, were often in close apposition in the same microscopic field, and transitions between the spindle and the round cells with slight development of fibrils were plentiful.

In a third, rapidly growing, and very vascular tumor (Plate XXXVI, Fig. 3; Plate XXXVII, Fig. 1) of the uterus the cells in places, especially where they were invading the wall of the uterus, showed well the fibrils characteristic of smooth muscle cells, but elsewhere, especially where the blood spaces were large, the cells were undifferentiated spindle-shaped cells with relatively little cytoplasm. Such a tumor could easily be mistaken for a very cellular fibrosarcoma, for it sometimes does occur in this situation.

It is well to bear in mind that slow and rapidly growing smooth muscle tumors can occur in other parts of the body than in the uterus, such as the skin and the kidney, for example, and that the rapidly growing ones can readily pass through the hands of a pathologist, on routine hurried examination, as a spindle cell sarcoma without a suspicion of its true nature. This I know from personal experience.

The cells of the *neuroglia tissue* occur in two forms as ependymal cells lining the neural canal and the ventricles and as glia cells which are derived from the ependymal cells and lie between and support the elements of the central nervous system. The glia cells have spherical nuclei and produce characteristically staining fibrils which run along the surface of the cytoplasm of the cell to which they belong and extend out from the cell in all directions. Weigert was unable to determine definitely whether or not the ependymal cells produce fibrils, but it is probable that, with the exception of those covering the choroid plexus, they do.

In gliomata the cells show great variations in size and shape, but they always tend to differentiate like their normal prototypes, the ependymal and glia cells. The cells may be large or small, spherical or spindle-shaped, with few or many, fine or coarse fibrils. Gland-like cavities lined with ependymal-like cells occur in many of these tumors. All these facts in regard to gliomata are so well known that I shall limit my remarks to two very unusual cases which illustrate the value of the differential stains for fibrils.

The first case has already been referred to in print, but merits mention again in this connection. It was a tumor the size of a baseball occurring over the coccyx in a woman forty-four years old. For a year it had been growing rapidly. Before that time it had existed for twenty-five years to the patient's knowledge as a nodule the size of a hickory nut. Nearly a year and a half after the first operation the patient was operated on for metastases in the right groin. A year later a third operation was performed for recurrences over the coccyx and in the right groin and for metastases in the left groin. The patient died less than a year afterwards with recurrences in all three situations and with gradually increasing solidification of one lung suggesting metastases in it but no post mortem examination was obtained.

The original tumor and the recurrences all presented the same gross appearances: large and small distinctly encapsulated nodules usually closely bound together. The nodules for the most part

were fairly firm and elastic, but a few were somewhat soft. On section the surface was grey, translucent, and for the most part homogeneous. In some of the larger nodules, however, there were irregular areas of hemorrhage and of necrosis.

While the tumor in gross suggested the appearance of a sarcoma, the structure histologically is that of a carcinoma (Plate XXXVIII, Fig. 1). It consists of large and small alveoli of cells of an epithelial type embedded in a fairly abundant connective tissue stroma. In the most rapidly growing parts of the tumor the cells in the alveoli are often round, but as a rule they are more or less oval or spindle-shaped. The outlines of the cells are usually not very sharply defined. The nuclei are round to oval in shape, vesicular in type, and stain rather lightly. They contain numerous fine and a few coarse chromatin granules, but no distinct nucleolus. Mitotic figures (Plate XXXVIII, Fig. 3) are numerous.

The remarkable feature about this case is that fibrils are present in varying number between the cells which fill the alveoli. In some places they are very abundant, but in others they are few in number. They vary in thickness, but for the most part are rather coarse. Some of the fibrils are straight, but many of them are wavy.

Sometimes the cells in the peripheries of the alveoli immediately adjoin the connective tissue stroma, but often they are separated more or less from it by a layer of their own fibrils. Sometimes these fibrils run parallel with the stroma, at other times they lie perpendicular to it and terminate in swollen ends which unite laterally to form a sort of limiting membrane, just as the neuroglia fibrils do in the spinal cord of the embryo. The fibrils in the alveoli always run parallel with the long axis of the cells to which they belong.

In some of the tumor nodules the tumor shows another type of growth, namely, it becomes distinctly papillary. The cells in such cases are arranged in one or more layers around delicate papillary stalks containing blood vessels and a little connective tissue. The nuclei are in the ends of the cells farthest away from the vessels. Between the cells are a few fibrils radiating out from the central stalk.

In the smallest inguinal lymph nodes (Plates XXXVIII, Fig. 2) which were invaded the tumor cells extend along the peripheral sinuses in exactly the same way that metastases of carcinoma do, but everywhere they produce their own kind of fibrils.

This tumor probably originated from remains of the neural canal over the coccyx. These remains, in the form of gland-like cavities, can be demonstrated to be present in most, if not all, embryos as late as the fourth or fifth month, and probably persist in many to a much later period.

This case illustrates the close analogy existing between gliomata and epithelial tumors, in manner of growth, of metastases, and of malignancy. In another glioma, situated in the fourth ventricle, there was found a somewhat similar alveolar arrangement of the cells with reference to the stroma, and combination of ependymal and glia cells.

The second case came to post mortem examination under Dr. H. C. Low at the Children's Hospital and has not yet been reported in detail. The cause of death was a glioma originating in the lumbar region of the spinal cord. It had infiltrated and destroyed the lower part of the cord and then spread to the pia and along it, forming a thick layer surrounding the cord (Plate XXXIX, Fig. 1) throughout its entire length. It had also extended in the meshes of the pia all over the cerebellum (Plate XXXIX, Fig. 2) and cerebrum. Macroscopically the lesion resembled a thick inflammatory exudation with more or less organization, or possibly a diffuse tuberculous process, and was so diagnosed.

Microscopically the tumor is composed of rather large cells (Plate XXXIX, Fig. 3), for the most part of spindle shape. In places, however, the cells are more spherical and among them occur large multinucleated cells. Mitotic figures are numerous. The cells are surrounded by fibrils of but one kind and these stain in the same manner as neuroglia fibrils.

In places the tumor has invaded the upper part of the cord and the pons, but it is especially over the cerebellum that the invasion of the underlying tissue is most evident. Here the cells have spread everywhere between the leaflets of the cerebellar tissue and have infiltrated the molecular layer, and in places have destroyed the Purkinje cells and invaded the granular layer. Over the cerebrum both the growth of the tumor in the pia and the invasion of the underlying tissue are less marked.

This case, like the preceding one, illustrates the possibility of gliomata extending and invading tissues like other malignant new growths.

In regard to the *epithelial tumors* I am unable at present to make any special contributions. This group of tumors is large and it requires a long time in which to obtain type specimens of each variety, preserved under the best conditions. Unquestionably these tumors should be classified so far as possible, like their normal prototypes, according to the differentiation of their cells and secretions.

The phosphotungstic acid hematoxylin stain is useful in studying them not only because it brings out with great distinctness the nuclei and centrosomes as in other cells, but also because it stains the fibrils which occur in the normal epidermis and in those tumors of which the cells tend to differentiate like the epidermal cells. It also stains the fibrils (Plate XL, Fig. 1) which sometimes occur, for example, among the epithelial cells in cancers of the breast. In addition to these fibrils it brings out sharply the cuticular membrane of epithelial cells on the side adjoining a lumen and on that account may prove an aid sometimes in distinguishing the cells of certain epithelial tumors from endothelial cells which never exhibit such a membrane.

A few of the epithelial tumors already studied deserve, perhaps, brief mention, as they suggest what more careful histological study of this class of tumors may yield.

The epithelium lining the ducts and glands of the breast and the coil glands of the skin are surrounded by a layer of spindle-shaped cells which run parallel with the long axis of these structures. These cells are better developed and more numerous in some situations than in others and show definite fibrils of the myoglia type; that is, the fibrils tend to fuse at the spindle-shaped terminations of the cells so as to form what appear to be coarse fibrils.

In all types of benign adenomata of the breast these same cells with fibrils are present behind the lining epithelium and usually can be readily demonstrated but sometimes are poorly developed. As soon, however, as the epithelium of the breast takes on malig-

nant properties and begins to invade the surrounding tissue this row of smooth muscle cells disappears although the type of tumor may be typically glandular, and the epithelial cells then abut directly on the connective tissue of the stroma.

In epidermoid carcinomata the development of epidermal fibrils is often more extensive than in the normal skin and resembles closely the abundant formation of these fibrils often found under conditions of inflammation. In the type of carcinoma of the skin called non-cornifying by Ribbert and popularly known at present as carcinoma basocellulare, epidermal fibrils are usually present in small numbers as well as small, poorly developed epithelial pearls. The cells of this type of carcinoma resemble in their more or less elongated shape, small amount of cytoplasm and slight production of fibrils, the epithelial cells of hair follicles more than those of the surface epidermis and may represent differentiation like them.

In carcinomata of the breast straight and wavy fibrils (Plate XL, Fig. 1) are not infrequently found singly and in small clumps running between the epithelial cells. They are in no wise connected with the fibrils in the surrounding stroma. In the alveoli of some of the carcinomata of the breast numerous small cavities form between the cells and the edges of these cells develop a distinct cuticular membrane.

The stroma of carcinomata shows more of interest with the two staining methods employed than the epithelial cells themselves. As is well known the stroma is sometimes proliferating very actively and contains numerous fibroblasts in which mitoses can occasionally be found. In other cases the cells of the stroma show little or no activity and consist of a few fibroblasts surrounded by many collagen fibrils. Often these two conditions are found closely associated in the same tumor. When the fibroblasts of the stroma are active, fibroglia fibrils (Plate XL, Figs. 2 and 3) are found in great abundance; in the dense often hyaline parts they are few in number.

Occasionally great masses of fine and coarse elastic fibrils surround the ducts and the blood vessels in cancer of the breast. In the midst of these masses occur a few irregular cells surrounded by fibroglia fibrils. It would scarcely be justifiable, however, to conclude from this relation that these cells have developed the mass of elastic fibrils around them.

In a case of chorionepithelioma (Plate XLI, Figs. 2 and 3) of the uterus, where a diagnosis had been made and an assistant was able to be present at the operation and preserve the tissue immediately after removal, nothing in the way of fibril formation could be found in the tumor cells, but in the smooth muscle cells, especially those nearest the tumor, the myoglia fibrils were unusually numerous and coarse.

In one instance of cancer of the breast in which proliferation was very active the tumor cells seemed to have gone wild. Many of them were very large and often multinucleated. Single and multiple mitoses were present in great numbers. The centers of some of the large alveoli were hollowed out and filled with fluid in which cells in mitosis floated free. In places the stroma between the alveolar masses was invaded and overwhelmed by the tumor cells which were unusually large and showed many mitotic figures. Such places might suggest to some the transformation of a carcinoma into a sarcoma.

Another tumor, very recently received, bears more directly on the question recently raised in connection with the inoculable tumors of rats and mice, of the possibility of the transformation of a carcinoma into a sarcoma. The case is one of solid tumors of both ovaries with metastases in the omentum. Sections show in places a typical epithelial type of growth; definite alveoli filled with cells and in places glands lined with cuboidal epithelium. In other places the cells are of spindle type and are arranged in bundles which run in all directions. These cells give rise to fibroglia and collagen fibrils. Among these cells are other large, pale cells with excentrically situated nuclei and with the cytoplasm transformed into a pale homogeneous material. The tumor probably represents an embryoma with slight differentiation of its cells, possibly the mesoblastic layer alone with the cells differentiating in part like the mesothelium, in part like the mesenchyma.

The study of certain epidermoid carcinomata, especially of the tongue and lip, are of interest in connection with the question of the possibility of the disappearance and cure of these tumors. Two cases in particular show marked invasion of many parts of the tumor by endothelial cells (Plate XLI, Fig. 1), many of which

have become transformed into foreign body giant cells. They are attracted by the cornified epithelial cells which they incorporate and gradually dissolve. The tumor cells in these areas often disappear and there then remain large masses of endothelial and giant cells. They, together with more or less reaction on the part of the surrounding connective tissue, often present a picture which resembles more or less closely tuberculous tissue or even a giant cell sarcoma.

The tumors arising from *endothelial cells* have proved interesting from the fact that these cells possess negative rather than positive characteristics, and yet they have some distinguishing features of their own. By endothelial cells I understand those cells of mesenchymatous origin which line the blood and lymph vessels, the inner surface of the dura and outer surface of the pia. They are flat cells with oval nuclei, a moderate amount of cytoplasm, and no intercellular substance; in other words they are not highly differentiated structurally and have little to characterize them. For this very reason, however, the cells stand out in marked contrast to strongly characterized cells, such as the fibroblasts and the smooth muscle cells.

Three groups of tumors arising from endothelial cells are recognized, (1) blood vessel, (2) lymph vessel, and (3) dural endotheliomata. Under these terms I include the angiomata because the endothelial cell is the only essential cell in all these tumors, and there is no reason for making an artificial division and considering separately the slow-growing ones in which definite vessels are formed (the angiomata), and those in which the cells grow rapidly and sometimes in solid masses (the endotheliomata). Such a separation tends only to complicate and confuse the subject.

Tumors arising from blood-vessel endothelium, the hemangioendotheliomata, are of fairly common occurrence, especially the capillary type, and some of them show active growth and extension. Their characteristic manner of invading fat tissue, their frequent extension into muscle tissue, and the way in which they surround the sweat glands are well known. It is to certain other interesting features that I wish to call attention here.

In the capillary angiomata the endothelial cells often line the vessels two to four layers thick and mitosis may occur in the cells

even in the outer layers. This proliferation causes narrowing of the lumen of the vessel and sometimes complete occlusion. In this way concentrically arranged masses or whirls of endothelial cells are formed. These clumps of cells are then slowly invaded by collagen fibrils from the surrounding connective tissue and gradually transformed by compression into flattened elongated cells which resemble fibroblasts, but they produce no fibroglia or other kind of fibrils. A somewhat analogous condition is seen in acute desquamative glomerulonephritis where the crescents of epithelial cells are invaded by connective tissue cells and fibrils and gradually replaced by them. This proliferation of endothelial cells and occlusion of the lumen was particularly well shown by a hemangioma of the eyelid which extended to the orbit and invaded the eyeball. For an opportunity to study this case I am indebted to Dr. F. H. Verhoeff, of the Massachusetts Charitable Eye and Ear Infirmary. In this tumor the vessels are of larger size than usual, though distinctly of the capillary type. They have invaded many of the nerves, destroying some of them partially, others completely. Numerous mitotic figures (Plate XLIII, Fig. 3) prove that the endothelial cells are proliferating rapidly. In many places the endothelial cells are two to four layers thick around the lumen, which may be much narrowed or even occluded. In other vessels masses of endothelial cells project into the lumen (Plate XLIII, Fig. 2). Collagen fibrils may be seen gradually extending in from outside the vessel and forming a backing or support for the endothelial cells. In this way a concentric perithelial arrangement of cells is presented, but the growth is entirely from the endothelium within, not from a theoretical perithelium outside.

In a second hemangioma forming several discrete nodules in the lower half of a leg and clinically so painful that it was supposed to involve the nerves, the growth slowly returned in the same nodular form and was removed a second time four years after the first operation. The nodules consist of masses of blood vessels (Plate XLIV, Fig. 1) with walls composed of endothelial cells four to eight layers thick. The lumina are all small. In places the vessels are packed so closely together that the separate vessels are made out with difficulty. There is very slight reaction on the part of the

surrounding connective tissue and little or no extension of collagen fibrils in between the endothelial cells. This tumor is interesting for three reasons, the thick walls composed of endothelial cells already mentioned, the fact that in a few places the cells on the outer surface of the vessels are extending into the surrounding fat and connective tissue, and finally because in places the new formed vessels are growing and extending inside of arteries (Plate XLIV, Fig. 2) and veins. In the latter it causes marked dilatation of the vessel wall.

Apparently closely related to this tumor is a group of three small new-growths, two of which occurred in the bend of the elbow and were exceedingly painful on pressure. So far as can be made out they are slow-growing hemangiomata in which the endothelial cells have extended outside of the walls of the vessels and have invaded the surrounding tissue to a greater extent than in the case just mentioned.

Another case studied consisted of the original nodular growth on the back of a youth of sixteen and of three recurrent nodular masses. Each time on routine examination a diagnosis of spindle cell sarcoma was made. Most of the tumor tissue (Plate XLII, Fig. 1) presents that type of growth. In places, however, all of the nodules show on careful microscopic examination appearances which arouse attention. These consist of large and small concentrically arranged clumps of cells (Plate XLII, Fig. 3) and also in a few places of irregular branching masses of cells (Plate XLII, Fig. 2). The solution, in regard to the nature of the nodular masses and in regard to the source of the recurrences, lies in the fat tissue surrounding the nodules. This is being invaded in many places by capillary blood vessels (Plate XLIII, Fig. 1) with prominent endothelial cells in which mitosis is frequent. In all the larger tumor masses, from outside pressure or from internal proliferation the lumina of many of the vessels have become occluded: as a consequence the circulation of the blood was interfered with, and vessels were no longer formed. Instead, the proliferating endothelial cells have formed clumps or whirls and irregularly branching and connecting masses of cells. These collections of cells have been for the most part gradually invaded by collagen fibrils and

transformed into flattened, elongated cells. There is no reason to assume that the endothelial cells take on new properties and produce the fibrils. The endothelial cell is a definite entity with characteristics of its own and it retains them under a great variety of conditions. In this class of tumors the collagen fibrils are always most abundant around the blood vessels where connective tissue cells are present, and from there spread out in diminishing numbers among the surrounding endothelial cells.

Two cavernous angiomata, both congenital and both increasing in size and spreading since birth, throw light on some points connected with the difficult subject of endotheliomata. In both cases the tissue was dropped instantly at the time of operation into Zenker's fluid and was not sectioned until the blood in the vessels was coagulated. The first tumor occurred in the form of ten separate projecting nodules on the shoulder of an eight-year-old girl. The nodules varied from one to four centimeters in diameter. Microscopically they consist of thin membranes of connective tissue covered with flat endothelial cells (Plate XLIV, Fig. 2). In places the membranes are packed closely together: in other places they are widely separated so that cavities of considerable size are formed. In several places the arteries in and around the tumor are filled with these same membranous folds of connective tissue covered with endothelium. The veins (Plate XLV, Fig. 1) are much more extensively invaded and often greatly dilated. Indeed the presence of narrow bands of smooth muscle cells at the edge of many of the larger masses of tumor suggest strongly that they too were originally within vessels but have broken through the walls in places.

This tumor shows three other points of interest: (1) A few small collections of endothelial cells concentrically arranged, apparently where a vessel lumen has been obliterated by a growth of endothelial cells. (2) An occasional starting point of one of the membranous folds: an outgrowth from a vessel wall of a collection of endothelial cells of cuboidal form with a little connective tissue to support them. (3) Numerous oval and spherical, more or less completely organized thrombi, with a single small pedicle to connect them with the wall. The thrombi consist almost entirely of fibroblasts (Plate XLV, Figs. 2 and 3); in a few of them an occasional small blood vessel can be seen.

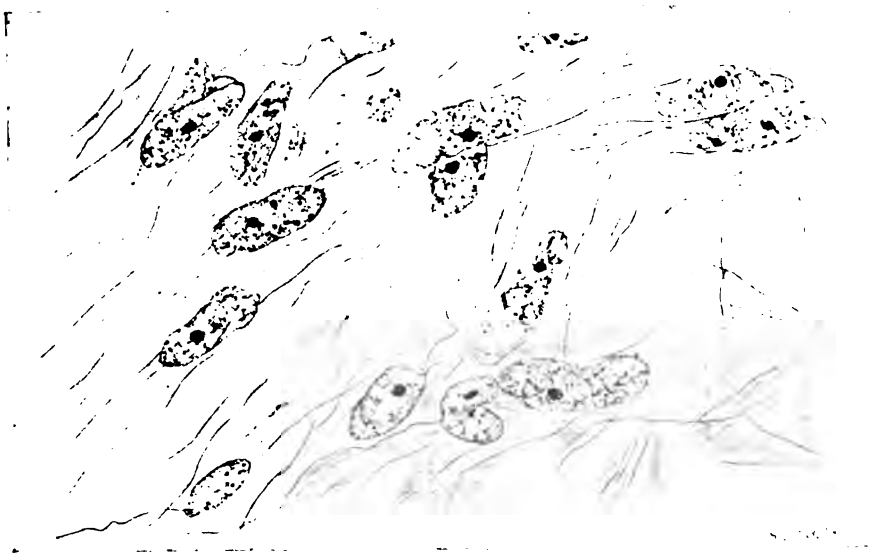
The second case was much more extensive and involved the right hand, arm, and shoulder of a girl (Plate XLVI, Fig. 1) of sixteen. Only three small nodules were removed, but in them the same growth within arteries and veins (Plate XLVI, Fig. 2), although to a less degree, was found.

This growth of endotheliomata within arteries and veins, with dilatation of the latter, throws light on the method of extension of these tumors and should prove of value clinically. It has been observed before, as far as I am aware, only in a case of rapidly growing endothelioma.

Lymphangioendotheliomata are rare and their diagnosis is usually not so positive and convincing as in the case of the blood vessel tumors where the contents of the vessels aid a great deal. I, therefore, report two cases with some hesitancy. The first, a rounded mass three centimeters in diameter was found subserous in a cornu of a uterus removed for a submucous leiomyoma. It consists microscopically of spaces lined with flat cells of the endothelial type and also of irregular collections and rows of cells of more cuboidal form, which often contain one to three large vacuoles (Plate XLVI, Fig. 3). These vacuoles seem to combine to form lumina around which the cells arrange themselves and flatten out as the vessels dilate. All stages between the solid masses of cells and the thin-walled vessel are present. This tumor has extensively invaded the muscle wall of the uterus.

The second case occurred in the left lumbar region beneath the muscles, but not connected with the kidney. It shows a similar condition, large dilated spaces lined with very low endothelial-like cells, and more cellular areas in which the cells are more or less spherical and often vacuolated. No mitoses were found in either case, indicating slow growth. The cavities contain a thin, serum-like fluid in which are a few lymphocytes.

Although the dural endotheliomata do not form vessels they have certain characteristics in common with the other endotheliomata. The cells grow in masses and form irregular whirls (Plate XLVII, Fig. 1) composed of flat cells closely packed together. In those tumors which are growing rapidly no intercellular fibrils are found between the endothelial cells. They occur only in connec-



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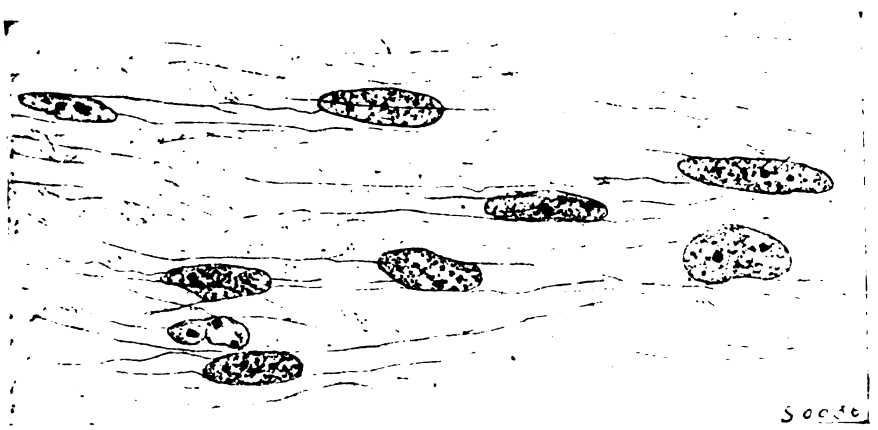

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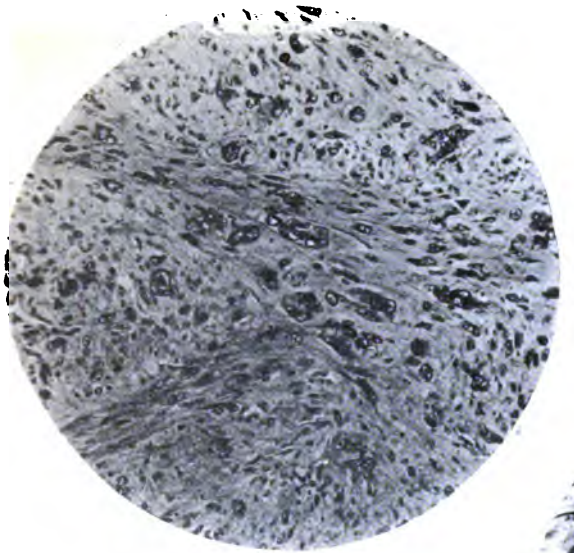

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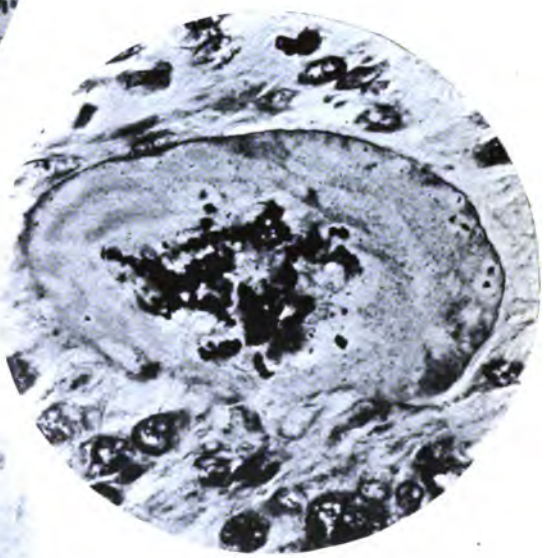

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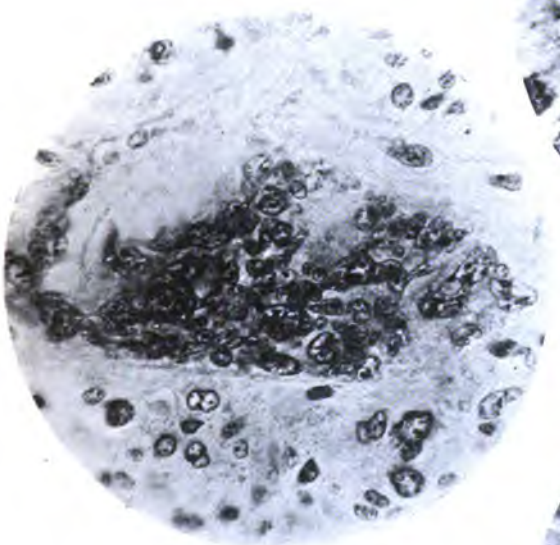

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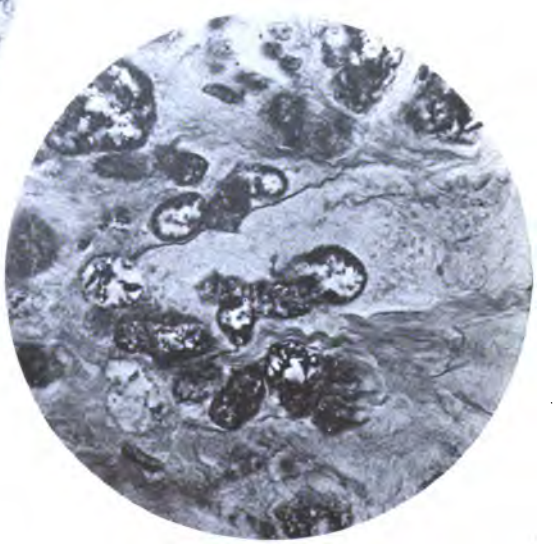

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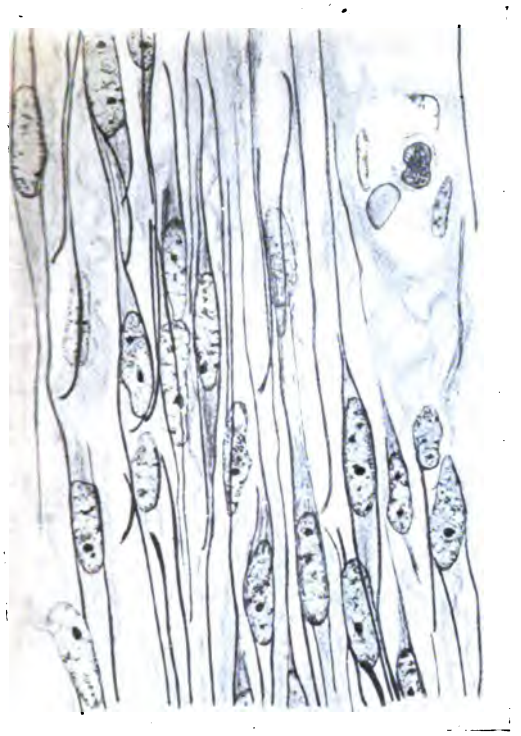

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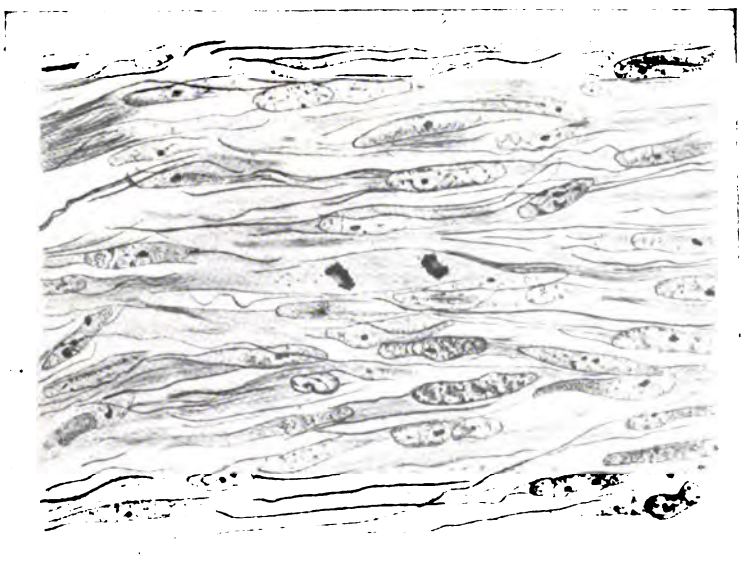

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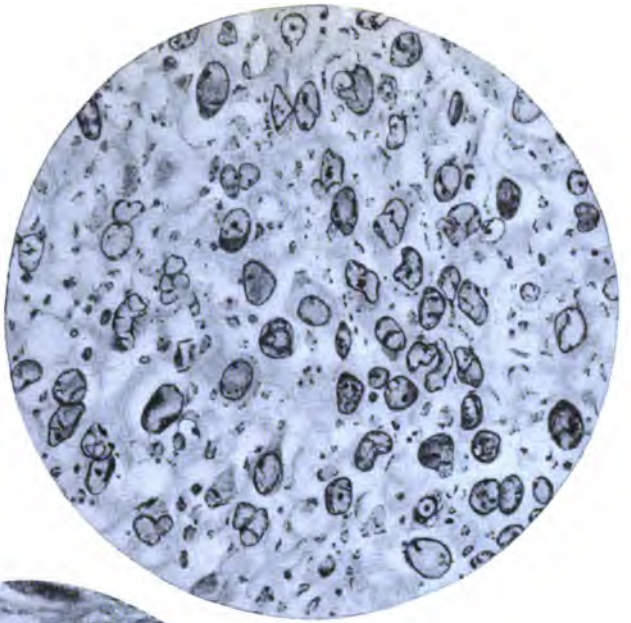

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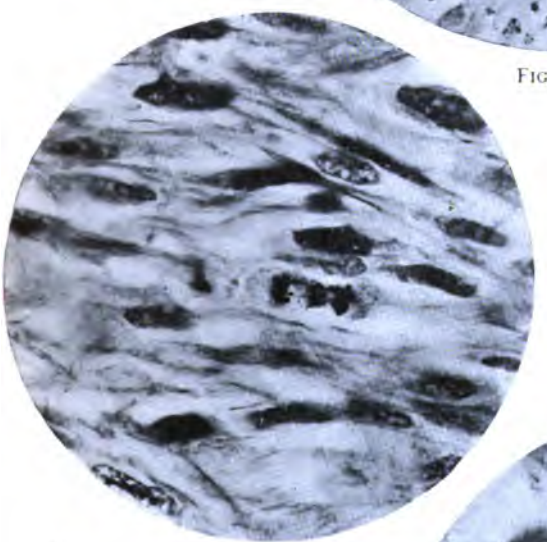

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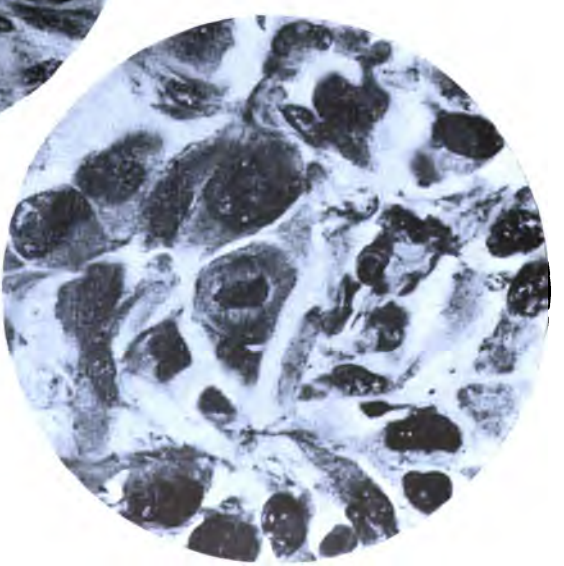

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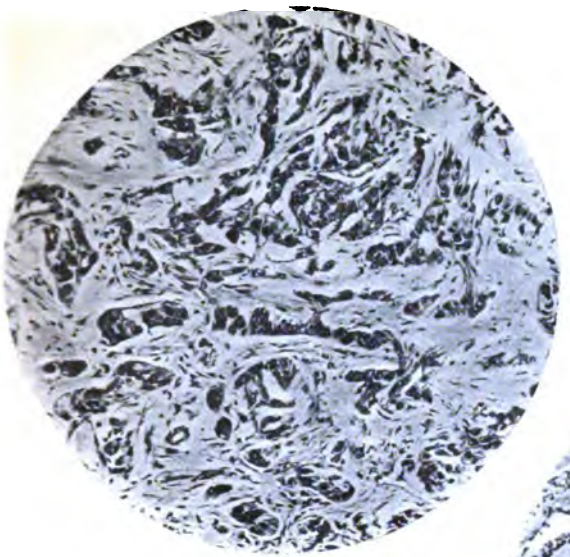

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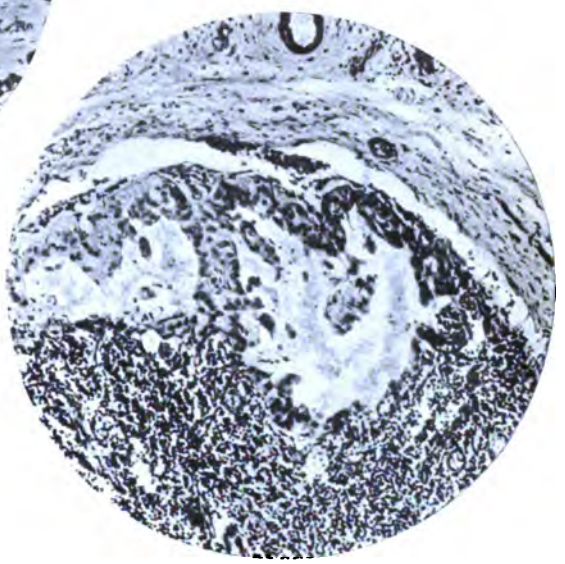

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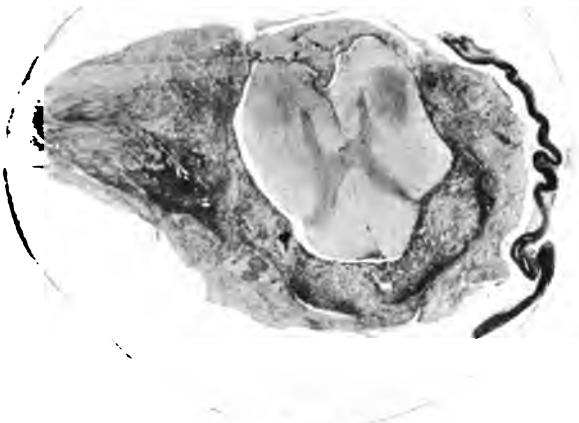

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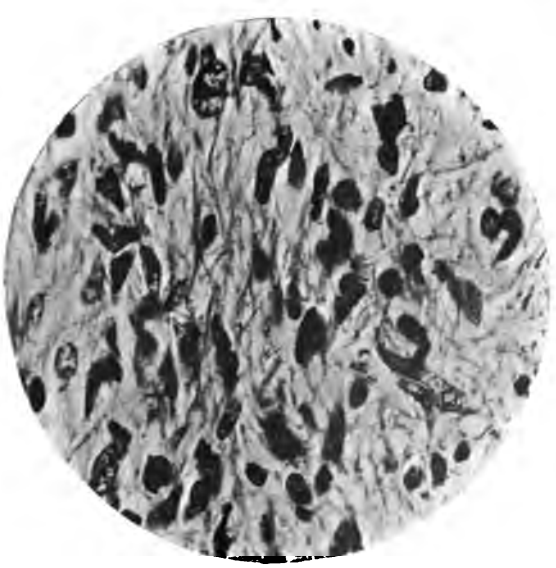

FIG. 3.

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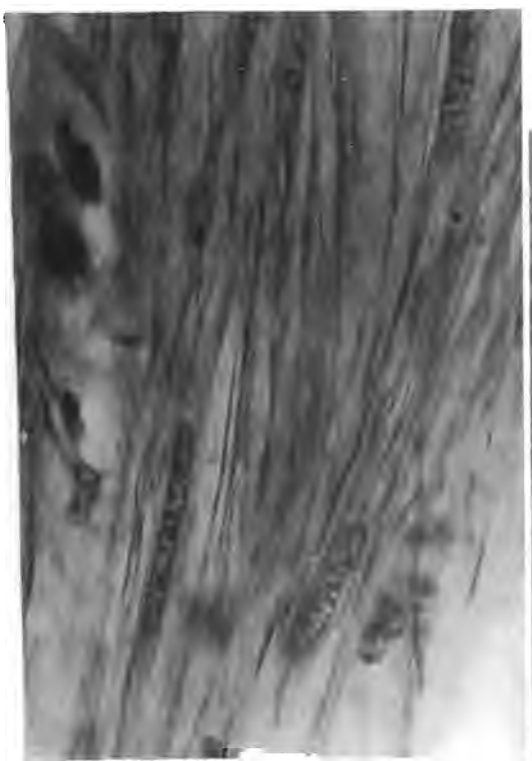

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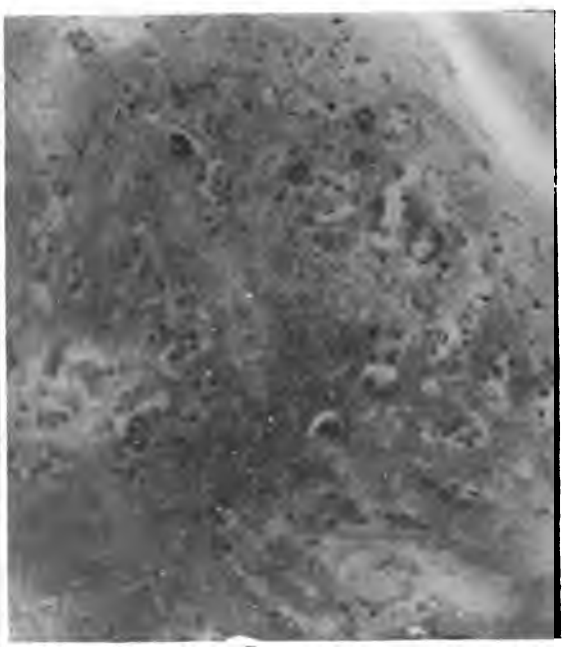

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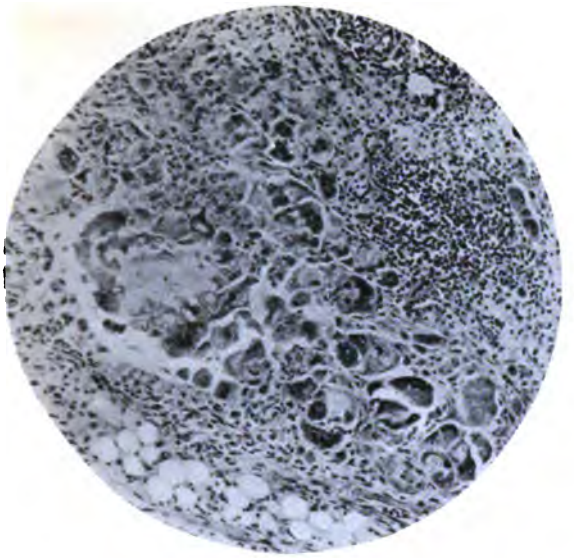

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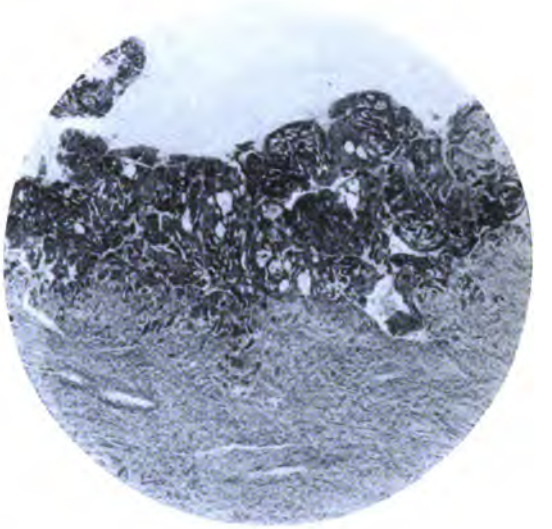

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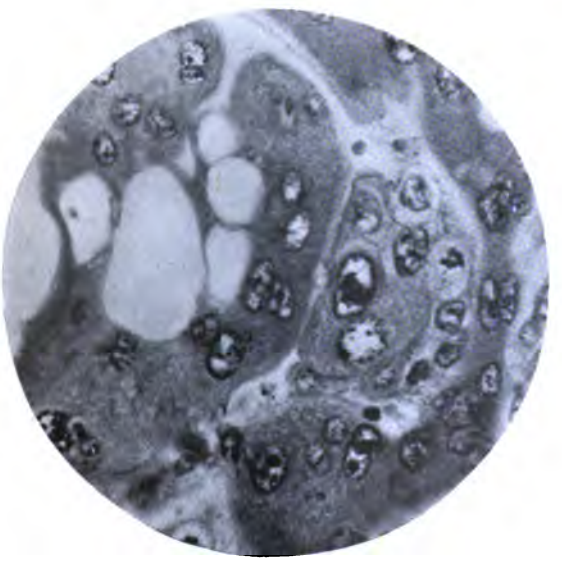

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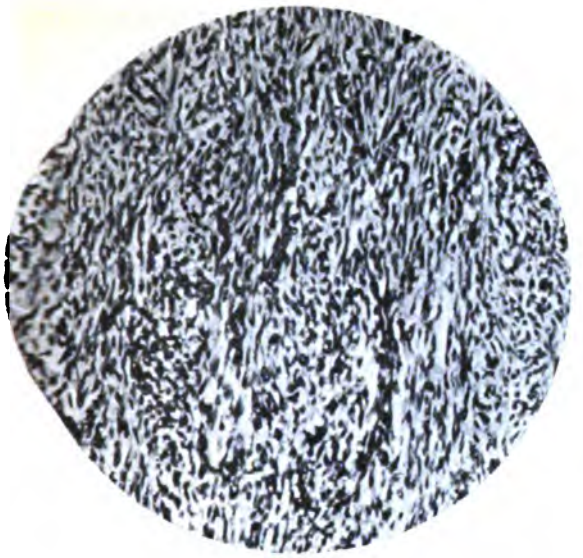

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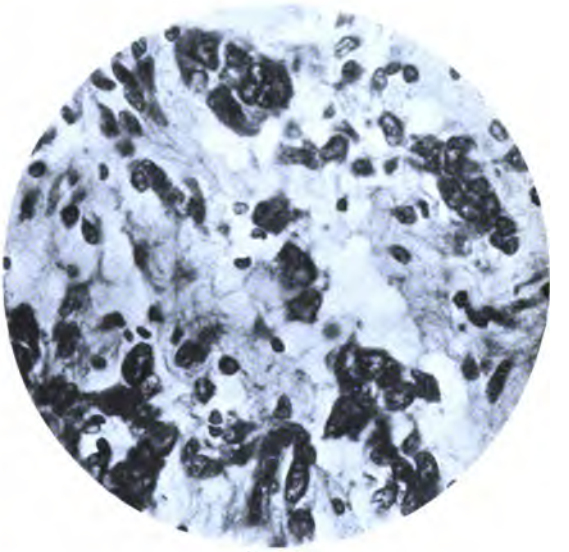

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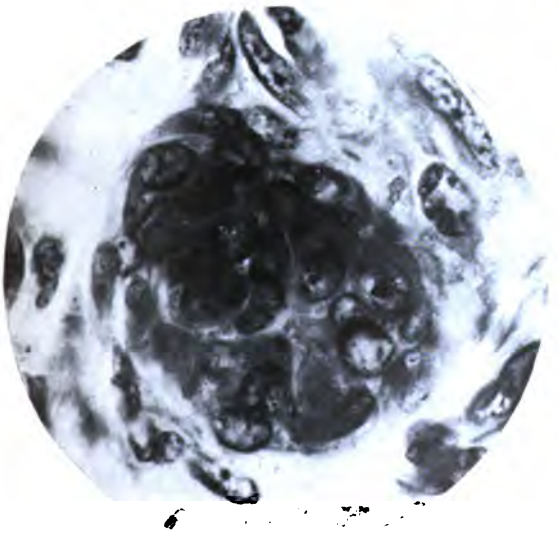

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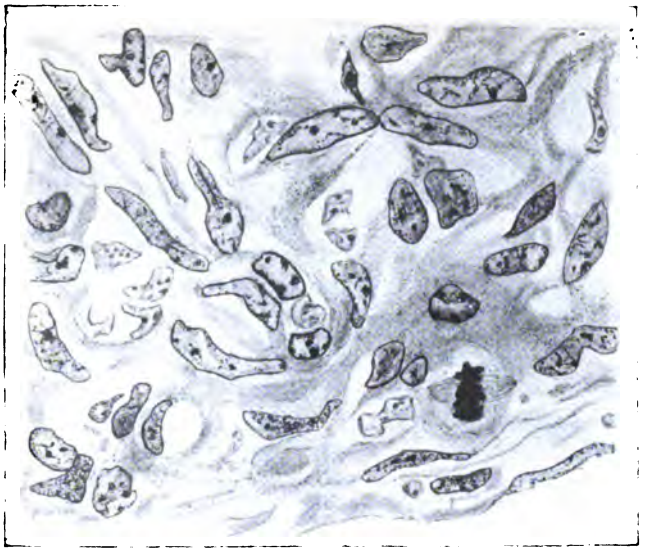

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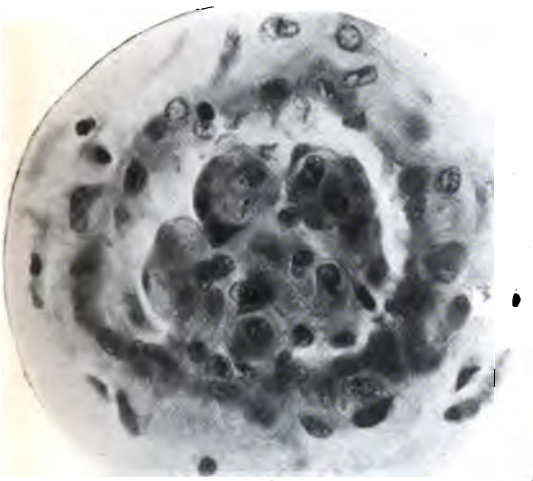

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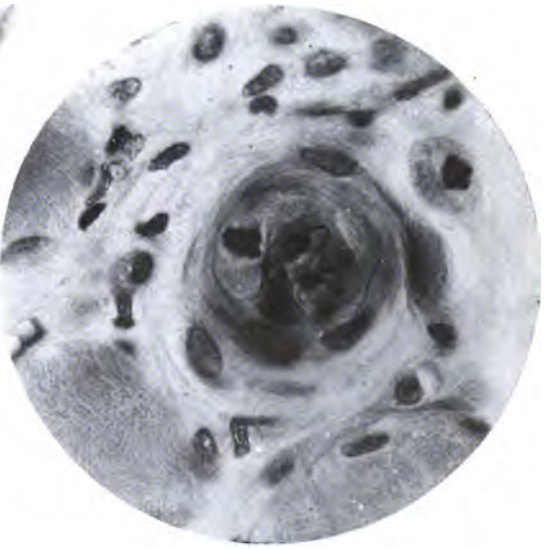

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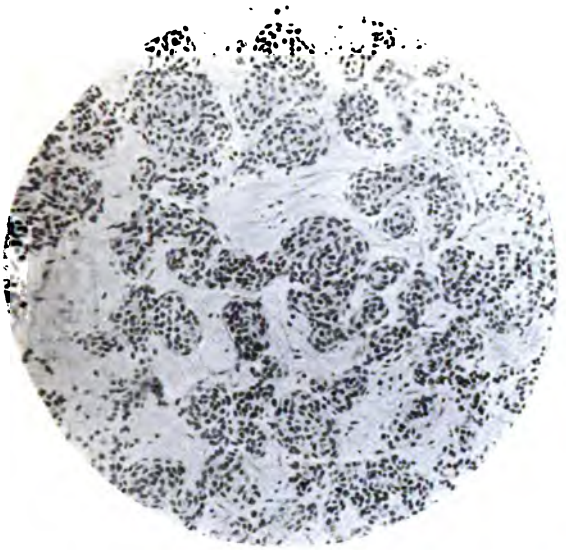

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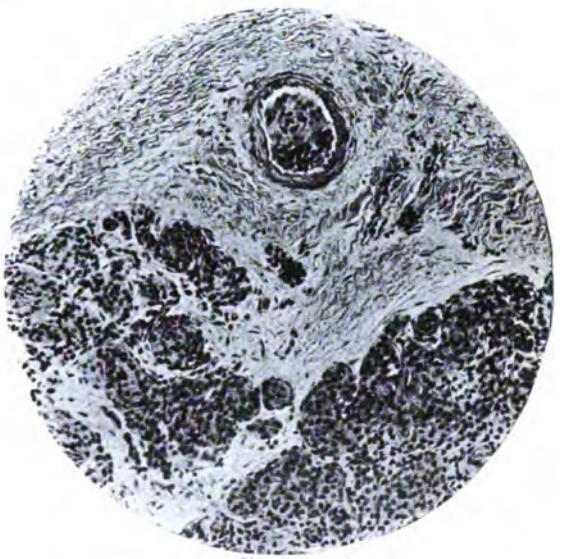

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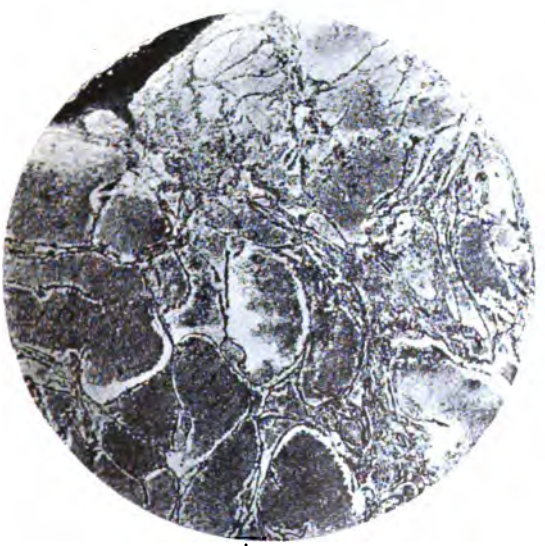

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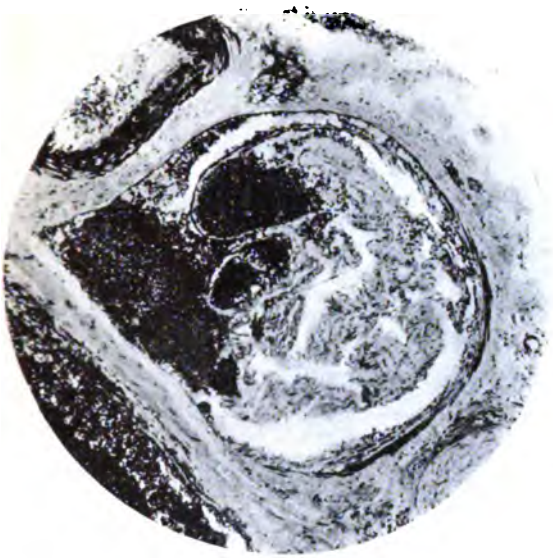

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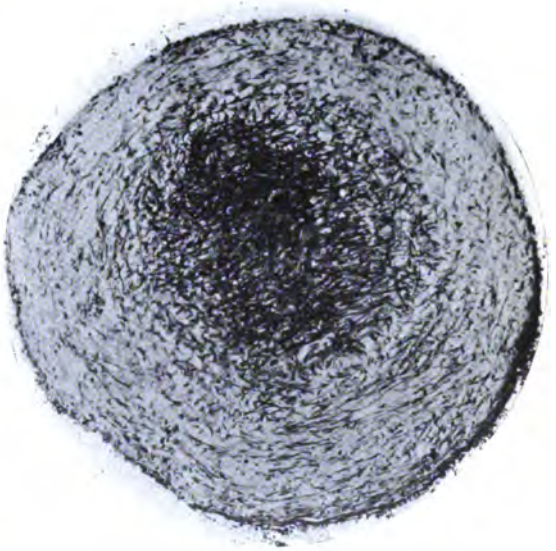

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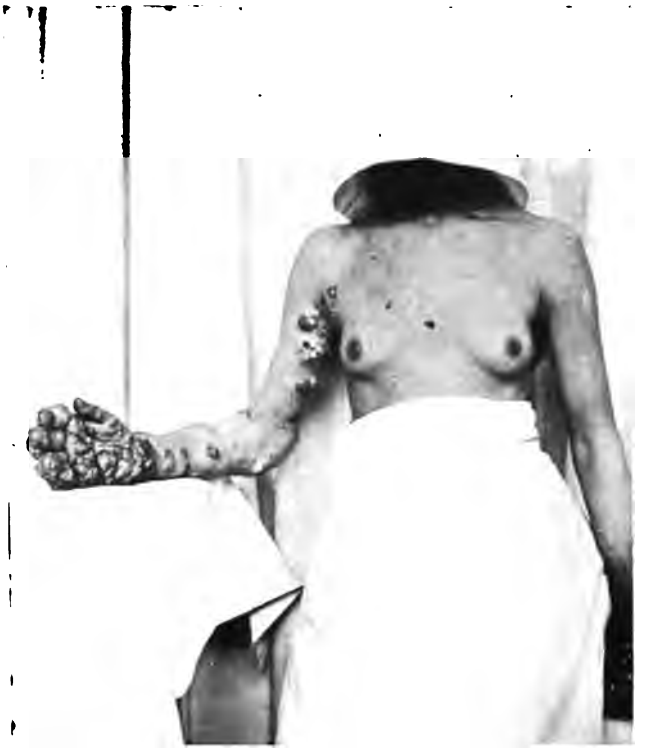

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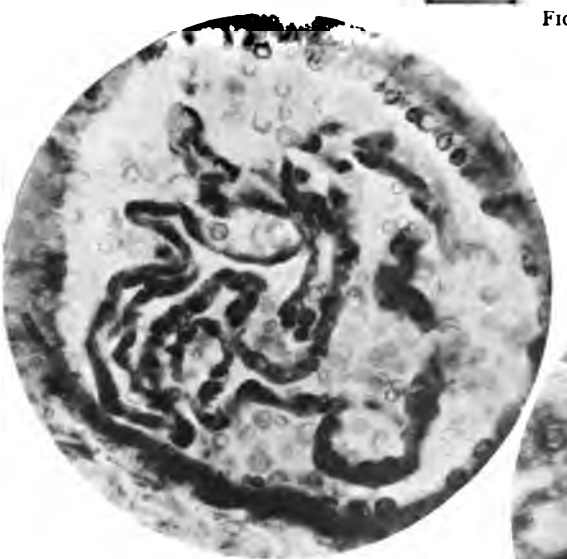

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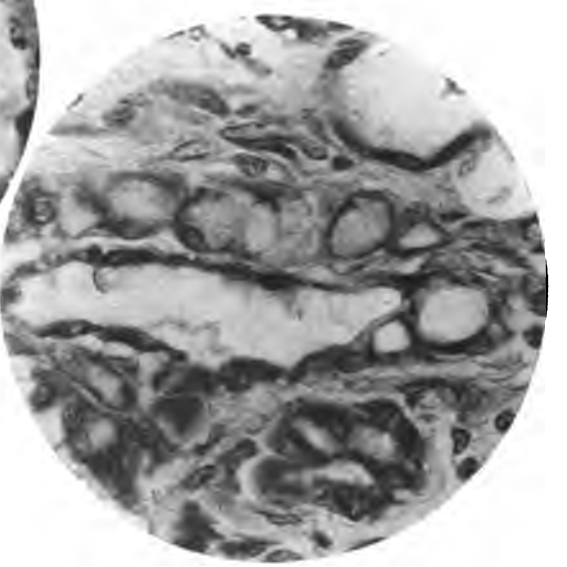

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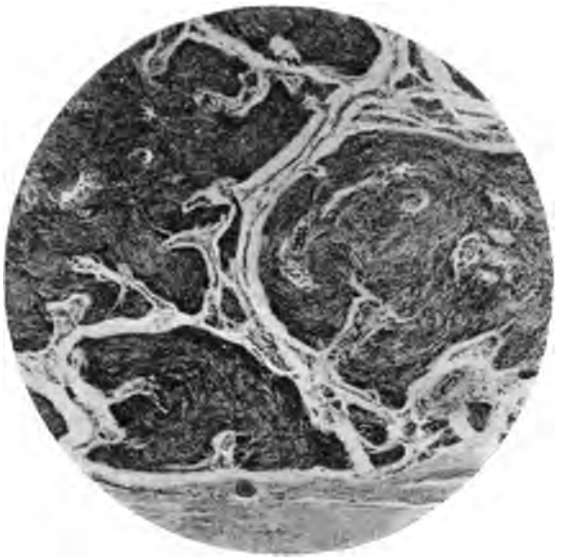

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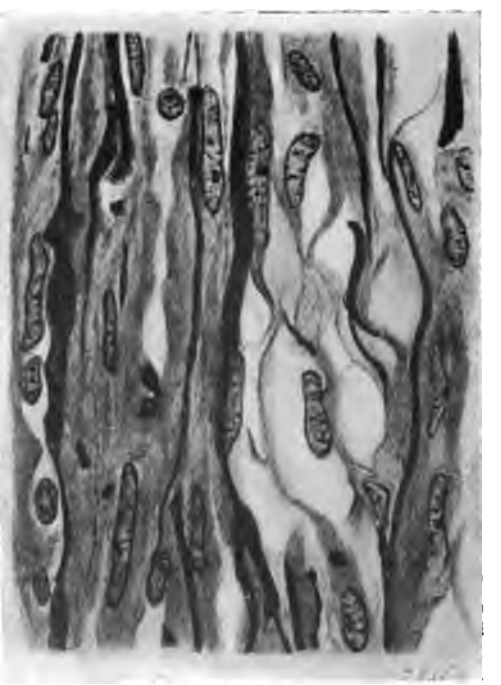

FIG. 3.

tion with the connective tissue cells around the blood vessels which run irregularly through the tumor-mass and form a stroma which nourishes and supports it. In the more slowly growing tumors (Plate XLVII, Figs. 2 and 3) a small amount of connective tissue, usually in the form of a reticulum of collagen fibrils, extends in between the layers of endothelial cells and forms a support for them. It is unquestionably derived from the connective tissue cells around the blood vessels.

I have not touched on all classes of tumors in this brief report, but I have made evident, I think, what I am striving at.

1. To determine more exactly than has been done heretofore how each kind of normal cell is characterized so that it can be definitely distinguished from all other cells, and then apply this knowledge to the study of tumors.

2. To determine the essential cell in each kind of simple tumor and name the tumor accordingly.

3. To do away, as far as possible, with all names of tumors in which accidental and secondary features are put first and the true nature of the tumor is lost sight of, such as the names round, spindle, and mixed cell sarcoma, perithelial angiosarcoma, psammoma, fibroendothelioma, etc.

In the lantern slide exhibit which follows, I am indebted to Dr. S. B. Wolbach for the photomicrographs of sections and to Miss Etta R. Piotti for the drawings.

The following illustrations represent a selection of forty-three out of the one hundred shown at the lecture:

EXPLANATION OF PLATES.

Plate XXXIV.

Figs. 1, 2 and 3 are from a fibrosarcoma and show fibroglia and collagen fibrils. The cells are seen flatwise in Fig. 1, sidewise in Fig. 2, and crosswise in Fig. 3.

Plate XXXV.

Figs. 1, 2, 3 and 4 are from a fibrosarcoma containing many multinucleated cells.

Fig. 2 shows multiple mitosis.

Fig. 3. A multinucleated cell with numerous centrosomes.

Fig. 4. A coarse fibroglia fibril running over the surface of a multinucleated cell.

PLATE XXXVI.

FIG. 1. An edematous leiomyoma showing what seem to be coarse myoglia fibrils.

FIG. 2 is a cross section from the same case and shows that the coarse fibrils are due to fusion of fine fibrils.

FIG. 3 shows a mitotic figure and coarse myoglia fibrils in a malignant leiomyoma.

PLATE XXXVII.

FIG. 1. Cross section of a malignant leiomyoma.

FIG. 2 shows spindle-shaped cells in another malignant leiomyoma.

FIG. 3 shows the spherical shape of cells in other portions of the same malignant leiomyoma.

PLATE XXXVIII.

FIGS. 1, 2 and 3 are from the case of glioma over the coccyx.

FIG. 1 shows the alveolar arrangement.

FIG. 2. Early invasion of an inguinal lymph node.

FIG. 3. A mitotic figure of a neuroglia cell within an alveolus. The bending of the neuroglia fibrils is an artefact due to the action of the knife in cutting the paraffin sections.

PLATE XXXIX.

FIGS. 1, 2 and 3 are from the case of glioma of the lumbar cord with extension in the pia along the cord and over the brain.

FIG. 1 shows the tumor surrounding the upper dorsal cord.

FIG. 2 shows the extension over the surface of the cerebellum.

FIG. 3 shows the histological structure of the tumor.

PLATE XL.

FIG. 1 shows the epithelial fibrils within an alveolus of epithelial cells in a cancer of the breast.

FIGS. 2 and 3 show the fibroglia fibrils of the stroma of the same case in longitudinal and cross section.

PLATE XLI.

FIG. 1 shows a large group of endothelial cells ingesting the cornified cells of an epidermoid carcinoma. Many of the endothelial cells are multinucleated (foreign body giant cells).

FIGS. 2 and 3. Low and high power views of a chorionepithelioma of the uterus.

PLATE XLII.

FIGS. 1, 2 and 3 are from a hemangioendothelioma of the back of a youth of sixteen.

In Fig. 1 the cells have the appearance and arrangement of a spindle cell sarcoma.

FIG. 2 shows irregular clumps of endothelial cells, and Fig. 3 a large whirl of endothelial cells.

PLATE XLIII.

FIG. 1. From the hemangioma of the back of a youth of sixteen. The figure shows the type of capillary vessels invading the fat tissue outside of the tumor nodules.

FIGS. 2 and 3 are from the hemangioma of the eyelid invading the orbit.

FIG. 2 shows masses of endothelial cells extending into the lumen.

FIG. 3 shows mitosis of an endothelial cell and almost complete occlusion of a vessel by proliferation of the endothelial cells lining it.

PLATE XLIV.

FIG. 1. Hemangioendothelioma of the leg and ankle; the figure shows the thick-walled vessels running in every direction. The endothelial cells of the walls are several layers thick and the lumina are exceedingly small.

FIG. 2 is from the same case and shows the growth extending within a small artery.

FIG. 3 is from the cavernous hemangioma of the shoulder and shows the characteristic type of growth.

PLATE XLV.

FIG. 1 is from the cavernous hemangioma of the shoulder and shows the tumor growing within and dilating a vein.

FIGS. 2 and 3 are from the same case and show low and high power views of an organized thrombus consisting entirely of fibroblasts.

PLATE XLVI.

FIG. 1. Photograph of a clinical case of cavernous hemangioma of the hand and arm.

FIG. 2. Cross section of a small vein from the same case showing the tumor growing within the lumen of the vessel.

FIG. 3. A lymphangioendothelioma of the uterus showing the hollowed-out cytoplasm of some of the cells and the flattened cells lining the larger spaces.

PLATE XLVII.

FIG. 1. A cellular, rapidly growing dural endothelioma. The only collagen fibrils are in the small amount of connective tissue accompanying the blood vessels which run between the masses or whirls of endothelial cells.

FIGS. 2 and 3 are from a slow growing dural endothelioma and show the reticulum of collagen fibrils which has extended in between the endothelial cells. In Fig. 2 the cells are seen flatwise and in Fig. 3 sidewise.

FURTHER STUDIES ON THE OPHTHALMO-TUBERCULIN REACTION IN CATTLE.[1]

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PLATE XLVIII.

Report on Cattle Used in First Series of Experiments (7).—In our previous experiments the cattle used were divided into three groups. Class A contained five animals which appeared to be in the best of condition with the exception of No. 2A, which was beginning to show the clinical signs of tuberculosis. These animals were tested with tuberculin by the ordinary subcutaneous method eight months before the ophthalmo-tuberculin test was applied. It was stated that all these animals gave a most pronounced ophthalmo-reaction when first tested. Class B, containing five animals, was secured from a herd of thirty-four cattle, twenty-seven of which reacted to the usual subcutaneous tuberculin test. This test was applied only one month previous to the application of the ophthalmic test. In all these animals, as stated in our previous paper, an ophthalmo-reaction was observed, but in a very mild form. We made the tentative statement that the recent subcutaneous tuberculin test might possibly have inhibited the subsequent ocular reactions in Class B, but also stated that since the animals showed themselves clinically to be in the advanced stages of tuberculosis the generalization of the tubercular toxins in the body might possibly inhibit the ophthalmo-tuberculin reaction as it does occasionally the usual subcutaneous test and, consequently, produce only a slight reaction. From our later experiments we are inclined to believe that the ordinary subcutaneous tuberculin does inhibit the eye reaction in a cer-

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tain percentage of cases (50 per cent.) which are instilled the first time. We are inclined to believe also that the more generalized or advanced the tubercular process is, the longer the eye will be in a state of anaphalaxis to the tuberculin. It may be also stated here that it is important to differentiate between the natural and induced hypersusceptibility of the conjunctiva. While the ocular reaction may be readily induced several times, it will not be as pronounced unless the conjunctiva has become artificially hypersensitized by spaced instillations. Further experiments were made on Class A and will be referred to later. Necropsies were held on Class B soon after our first experiments were made. (See Necropsy Report, Class B.) It will be seen on referring to the accompanying report of the necropsies that all the animals were affected with advanced tuberculosis. In our previous report we called attention to the finding of *Bacillus tuberculosis* in the fæces of No. 2B. We were able to demonstrate again the bacteria in the edges of the typical tubercular ulcers found in the large intestines of this animal.

Class C, containing twenty animals, was used as control. With the exception of No. 2C, all had been repeatedly tested with tuberculin by the usual method, the last time being six months before the ophthalmo-tuberculin test was applied. It was stated that No. 2C gave a very slight reaction to the ocular test. On being subsequently tested with tuberculin by the usual subcutaneous method No. 2C reacted, and on being necropsied showed tubercular lesions. (See Necropsy Report.) However, two other cattle among these controls, Nos. 8C and 10C, which did not react to the first ophthalmic test, on being subsequently tested with tuberculin by the usual subcutaneous method reacted to this test, as well as later to the ophthalmic test. On necropsy quite extensive tubercular lesions were observed. Undoubtedly these cattle were tubercular when the first ophthalmic test was applied. We can offer no explanation except that the conjunctiva of these cattle was not sufficiently anaphalactic or was antianaphalactic to tuberculin for some unknown reason, or what is more probable, that in these beginning tests our inaccuracy of observation was responsible for not seeing a slight reaction which possibly occurred. Both antianaphalaxis or immunity and very slight reactions of the conjunctiva have been observed during our recent experiments.

Cattle Used in Recent Experiments.—The cattle in Class A and Class C, and in addition a new group of tubercular cattle which we have designated as Class D, have been used. For the purpose of *control* we have used another group of twenty-six non-reacting cattle (temperature test), Class E. Classes D and E were last tested with tuberculin by the usual subcutaneous method in January, 1908.

Class D—Tubercular.

1. D Grade Shorthorn.
2. D Jersey.
3. D Red Polled.
4. D Jersey (heifer).
5. D Holstein (heifer).
6. D Holstein.
7. D Holstein.
8. D Holstein (heifer).
9. D Grade Shorthorn.
10. D Holstein.
11. D Polled Angus.
12. D Galloway (steer).
13. D Polled Angus.
14. D Shorthorn (steer).
15. D Holstein.

Class E—Non-Tubercular (Control).

1. E Red Polled.
2. E Red Polled.
3. E Red Polled.
4. E Red Polled.
5. E Grade Shorthorn.
6. E Shorthorn.
7. E Devon.
8. E Holstein.
9. E Holstein.
10. E Grade Red Polled.
11. E Red Polled.
12. E Red Polled.
13. E Jersey.
14. E Jersey.
15. E Guernsey.
16. E Guernsey.
17. E Shorthorn.
18. E Grade Shorthorn.
19. E Grade Jersey.
20. E Polled Angus.
21. E Shorthorn.
22. E Grade Jersey.
23. E Short horn.
24. E Jersey.
25. E. Guernsey.
26. E Holstein.

Description of Experiments.—We have used in our recent investigations, as we did in our first work, the tuberculin as supplied by the Bureau of Animal Industry, United States Department of Agriculture. This tuberculin is quite stable and well suited for comparative tests. The glycerine present aids in the absorption of the tuberculous material. The tuberculin was used full strength in the majority of cases. We were able, however, to elicit a more pronounced reaction in some few cases with this tuberculin concentrated to one tenth when the first instillation only gave a slight reaction. The glycerine or preservative agents were not responsible for the reaction. These cattle showed on necropsy a few small tubercular lesions.

In our first work we instilled 0.25 cubic centimeter into the conjunctival sac. We have since found that a smaller amount of tuberculin serves equally well. It has been our practice in these later investigations to use about one tenth of a cubic centimeter. Using this amount there is less chance of expulsion, and by slightly manipulating the lids the tuberculin becomes well distributed before lacrimation can become excessive. It was found to be advantageous to draw the lower lid down and instill the tuberculin on the inferior portion of the bulbar conjunctiva. Before making the instillation of tuberculin into the eye its condition was carefully compared with that of the opposite eye.

Reaction.—We have found that the ophthalmo-reaction varies greatly in different cattle. The reaction described in our previous communication (7) is typical of the rather severe form (see figures). Milder reactions often occur. Calmette (4) describes the reaction in man as a slight hyperæmia and swelling of the caruncle and conjunctiva, especially in region of the inner canthus. The reactions have been variously separated according to different writers. For example, Aubaret and Magne (2) and Aubaret and Laford (1) have separated the reactions into four divisions as follows: (*a*) mild form; (*b*) moderately severe form; (*c*) intense form; and (*d*) very intense form. In cattle we have divided the reactions observed into three divisions: (*a*) slight; (*b*) marked; (*c*) very marked. In recording the tests we have used a modification of the scheme suggested by Comby (5) and Baldwin (3):

(N) Negative = No difference observed in eyes.
(D) Doubtful = Slight redness of conjunctiva.
+ = Slight engorgement of palpebral conjunctiva with exudation.
++ = Marked engorgement of whole conjunctiva, photophobia, lacrymosis and exudation.
+++ = Very marked reaction.

Reactions of varying intensity were observed in all tubercular animals used in this group of experiments. Since our first work on this subject we have been able to distinguish in some cases a very mild form of the reaction which is characterized only by an engorgement of ocular vessels and no exudation (+). We have found also that the reaction reaches a maximum in from ten to twelve hours after the tuberculin is instilled.

REACTION CHART.

No.	Usual Tuberculin Test. 1	Ophthalmic Test (7). 1	Usual Tuberculin Test. 2	Ophthalmic Test. 2	Ophthalmic Test. 3	Ophthalmic Test. 4
1 *A*	Mar. '07+	Oct. '07+	Jan. '08+	+	+	*D*
2 *A*	Mar. '07+	Oct. '07+	Killed.	See Necropsy Report.		
3 *A*	Mar. '07+	Oct. '07+	Jan. '08+	++	++	+++
4 *A*	Mar. '07+	Oct. '07+	Jan. '08+	++	++	+++
5 *A*	Mar. '07+	Oct. '07+	Jan. '08+	++	++	+++
2 *C*	Mar. '07—	Oct. '07+(*D*)*	Jan. '08+	+	+	++
8 *C*	Mar. '07—	Oct. '07+(*D*)	Jan. '08+	+	++	++
10 *C*	Mar. '07-	Oct. '07+(*D*)	Jan. '08+	+	++	++

No.	Usual Tuberculin Test. 1	Ophthalmic Test (7). 1	Usual Tuberculin Test. 2	Ophthalmic Test. 1	Ophthalmic Test. 2	Ophthalmic Test. 3.	Ophthalmic Test. 4
1 *D*	Mar. '07—	x	Jan. '08+	++	++	++	+++
2 *D*	Mar. '07—	x	Jan. '08+	+	++	++	++
3 *D*	Mar. '07—	x	Jan. '08+	+++	++	*N*	*N*
4 *D*	Mar. '07—	x	Jan. '08+	+	+	++	+++
5 *D*	Mar. '07—	x	Jan. '08+	++	++	++	+++
6 *D*	Mar. '07—	x	Jan. '08+	+	+	++	o
7 *D*	Mar. '07—	x	Jan. '08+	+	+	++	+++
8 *D*	Mar. '07—	x	Jan. '08+	+	+	+++	+++
9 *D*	Mar. '07—	x	Jan. '08+	+	++	++	o
10 *D*	Mar. '07—	x	Jan. '08+	++	++	+++	o
11 *D*	Mar. '07—	x	Jan. '08+	*D*	+	++	o
12 *D*	Mar. '07—	x	Jan. '08+	++	++	+++	o
13 *D*	Mar. '07—	x	Jan. '08+	+	+	++	o
14 *D*	Mar. '07—	x	Jan. '08+	++	++	+++	+++
15 *D*	Mar. '07—	x	Jan. '08+	+	++	++	++

o = Killed before test was administered.
* = Reinstilled—first series.
x = Test not tried.

The tuberculin reaction, no matter how the tuberculin may be administered (ophthalmic, cutaneous, subdivided as cuti- and dermo-reaction, and subcutaneous) is due to the increased sensitiveness of the tissues of the tuberculous individual over the normal or non-tubercular. The hypersensitiveness has been termed anaphylaxis.

As pointed out previously, it has been known for some time that local reactions can be produced in infected individuals when the toxin from the organism causing the infection is introduced into

the body. Von Pirquet (9) exhaustively studied the phenomenon in the case of revaccination for smallpox and called particular attention to its significance. In the case of tuberculin injections it has frequently been noted that in tubercular individuals there was a marked hyperæmia and swelling at the focus of inoculation even where the injection was made with the strictest aseptic precautions. (Epstein [1891], Escherich, Spengler, Hamberger.)

The condition of hypersensitiveness which is present in tubercular individuals is an indication of an incomplete resistance on the part of the tissue cells of the body. This resistance does not produce complete immunity. When tuberculin is injected into an infected individual, at the focus of the infection a specific resistance is manifested and is characterized by hyperæmia and local leucocytosis.

Depending upon the extent of the tubercular process in the body and the amount of absorption of tubercular products, all the body cells become hypersensitive in varying degrees. The conjunctiva is a very delicate membrane, and is very easily affected by irritants. In other words the cells which compose it are normally extremely hypersensitive. In a tubercular individual the conjunctival cells are particularly sensitive to tuberculin or tuberculous material. We have here, as in all cells of the body, a condition of hypersensitiveness (anaphylaxis) and resistance combined. Von Pirquet has applied the term "*allergie*" to the condition of acquired immunity associated with anaphylaxis. As a result when tuberculin is instilled into the eye there is an attempt on the part of the conjunctiva to ward off the tubercular material. Consequently, there is a marked local hyperæmia and leucocytosis, with exudation in some cases. The intensity of the reaction will, as before stated, depend upon the extent of the tubercular processes in the body. In our investigations we determined that the local reaction is directly proportional to the extent of the tubercular lesions (compare Necropsy Report and Reaction Chart).

Undoubtedly the proteids derived from the tubercle bacillus are responsible for the reaction. Tuberculin is probably related to the nucleo-proteids, as is shown by the fact that it is destroyed by the tryptic enzyme, and by the slowness with which it is acted upon by pepsin. As would be expected, the washed proteid material from

ground-up bacteria of tuberculosis produces the local ocular reaction. It has been suggested that perhaps the beef proteids in the glycerine bouillon on which the bacteria were grown might influence the reaction. Our investigation demonstrates satisfactorily that this is not the case.

The instillation of full-strength tuberculin into the eye produced no serious results in the cattle used in our experiments.

Statement of Results of Last Series of Experiments; Ophthalmic Test.—It will be noted that the ophthalmic reaction occurred in varying degrees in all the tubercular cattle in Class D (see Reaction Chart). The reaction was most marked in those animals which showed on necropsy the most tuberculosis. This is, of course, different from the temperature findings in the usual subcutaneous tuberculin test which is usually most marked in those animals showing the least tuberculosis. Furthermore, it will be noted that in the majority of animals in Class D and also in Class A, which had been used in our first work, the reaction became more intense with subsequent instillations of tuberculin given at intervals of over two weeks, indicating a marked hypersensitiveness of the cells of the conjunctiva to tuberculin. In one case (No. 3D), when the four instillations were made at intervals of three days, the ophthalmic reaction did not appear after the last two instillations, indicating a local immunity (antianaphylaxis). We were unable to try the above experiment on any other cases. This animal showed on necropsy tuberculosis of both lungs and of the bronchial and mediastinal glands.

The Necropsy Report shows tuberculosis of the mediastinal lymph glands to be the most frequent locus of lesion.

We have found in a certain percentage of tuberculous animals, when a subcutaneous injection of two cubic centimeters of tuberculin is given at the same time that the eye is instilled, that the ophthalmo-reaction is much more severe. In our first work we were inclined to believe that a recent tuberculin injection inhibited slightly the ocular reaction. We find this to be true in only about fifty per cent. of the cases tested.

We have never observed in cattle the delayed reaction which has been reported in man by Montagon (8) and others. We did not

find the opposite eye to the one instilled hypersensitive, as has been reported on human subjects.

In Class E (controls), which were last tested with tuberculin by the usual method eight weeks before, one animal, No. 4E, twice showed a typical ophthalmic reaction (++). This animal showed no clinical signs of tuberculosis; it had been repeatedly tested by the usual subcutaneous method, and gave no reaction. This animal had previously received two instillations of tuberculin in the eye, and had failed to react. We believe that this reaction was a case of artificially induced local anaphylaxis. We were able in one case to induce purposely this local anaphylaxis to tuberculin by spaced instillations of tuberculin.

Result of Ophthalmo-tuberculin Test on Calves.—The dams (Nos. 2A, 4A and 5A) of these calves had repeatedly reacted to the usual tuberculin test and several times to the ophthalmo-tuberculin test. Necropsy showed the animals to have generalized tuberculosis (see Necropsy Report). The ophthalmic test was tried on these calves to determine if any conjunctival hypersensitiveness had been acquired *in utero*. The instillation of tuberculin in the eye failed to elicit any reaction whatever. Two subsequent instillations likewise produced no effect. The calves evidently were not in a state of anaphylaxis to tuberculin. It has been noted in guinea-pigs that the sensitizing substances can be transmitted to the fœtus *in utero,* and after birth the offspring give a reaction. It is possible that the calves were antianaphylactic or partially immune to tuberculin and tuberculosis. Subsequent experiments will be made with these animals.

It may be here stated in passing that No. 14D (shorthorn steer, one year old) was also the offspring of No. 2A. This steer on the first test made eight months after birth showed a slight reaction (+). Later the animal gave a pronounced reaction (+++). Necropsy on this animal showed tubercular lesions in both lungs, in the bronchial, mediastinal and inguinal glands, and in the intestines. It should also be noted that No. 2A had generalized tuberculosis with a tubercular abscess in the udder. No. 14D suckled No. 2A for four months and it was quite logical to expect intestinal tuberculosis in this animal.

Reaction to Ophthalmic, Cutaneous and Subcutaneous Tests Combined.—We were able to apply this combined test in only two cases. The eye was instilled and the subcutaneous and cutaneous tests were made as rapidly as possible in the foregoing order. About two minutes were consumed in applying these tests. In these animals the temperature curve was characteristic, but not high. The cutaneous reaction appeared in one of the animals, which was one year old, in about twenty-four hours and persisted for forty-eight hours. The reaction was characterized by a hyperæmia of the skin of the flank and later by an œdematous swelling. The ophthalmic reaction appeared in about eight hours and disappeared in twenty-four hours. There was not much fibrinous exudate in these cases. All three of these reactions were much lowered in their intensity.

Ophthalmo-tuberculin Test on Vaccinated Cattle.—Seven cattle were used in these experiments, six heifers and a young bull, of various breeds. These cattle had received intravenous injections of one, one and a half, and two cubic centimeters respectively of an emulsion of human tubercle bacilli in physiological sodium chloride solution. Guinea-pigs inoculated with the emulsion intraperitoneally died in four weeks. A second series of guinea-pigs died in five weeks. The cattle received three intravenous injections, six weeks apart. The method is that used by von Behring in Germany, and modified by Pearson in this country. When the ophthalmic test was applied to these vaccinated cattle it was noted that three gave on the first test a conclusive reaction which was not very pronounced (+) and four failed to react. On the second test after an interval of six weeks the three reacting cattle all showed an ophthalmic reaction of very low intensity (D). One heifer showed slightly more conjunctivitis than the others. Four animals failed to react. On the third test, six weeks later than the second, no reactions were observed on any of the cattle. When again tested in eight weeks, two of the three reacting cattle responded to the ophthalmic test. It would seem that at the time of the first test, as a result of vaccination, the three animals were in a state of anaphylaxis and as a result of the instillation of the tuberculin into the eye there was developed a condition of antianaphylaxis or local immunity. We

are inclined to believe that this condition of partially acquired immunity as a result of the ophthalmic test is entirely local, for when these three cattle were subsequently tested with tuberculin, by the usual subcutaneous method they gave temperature reactions. When first tested by the usual subcutaneous test these three cattle gave typical tuberculin temperature reactions. When tested again in six weeks, two showed an atypical reaction and one failed to react. Four animals failed to react to the usual subcutaneous test. Pearson explains this fact by stating that the animals subsequent to vaccination are in a condition of hypersusceptibility to tuberculin (anaphylactic). This hypersusceptibility is present for varying lengths of time. It requires, therefore, several injections of tuberculin in those animals which are still hypersensitive, to use up or combine with this hypersensitive substance (anaphylactin).

We are not prepared to state whether all these vaccinated animals are tubercular or entirely immune to tuberculosis. We will investigate this matter by necropsy as soon as possible.

Cutaneous Reaction.—Our experiments with this reaction alone have been quite limited. Only five animals were used. The skin on the flank was shaved and the tuberculin rubbed in. In from twenty-four to thirty-six hours a slight hyperæmia followed by œdematous swelling developed at the locus of inoculation in two cases. Subcutaneous injection of tuberculin by the usual method into the other three animals preceding another cutaneous test failed to elicit any cutaneous reaction. A typical temperature reaction was given in these cases. The cutaneous reaction is very difficult to observe on dark-skinned cattle.

We are, obviously, not prepared to state our views in regard to the efficiency of the cutaneous test on cattle at the present time. A distinction has been made by some writers between two methods of applying this test in man which are not applicable to animals. The term (cuti-reaction) is applied to the reaction following the rubbing of tuberculin on the shaved area of the skin and the term (demo-reaction) for the reaction which follows the application of tuberculin after the scarification of the skin. Whenever the thick skin of an animal like an ox is shaved there is, in a large majority of cases, some slight scarification, perhaps not easily visible. The

test, therefore, is made in the majority of cases after scarification. Further study will be given this test.

CONCLUSIONS AND SUMMARY.

Our conclusions differ in a few points from those of our first work on this subject. We are able to sum up our observations in this series of experiments as follows:

1. The ophthalmo-tuberculin test is of limited value in the diagnosis of tuberculosis in cattle. In some cases the reaction is very slight (hyperæmia). In others more pronounced congestion with profuse exudates are noted. Accuracy of observation is important. We are inclined to *rely primarily on the results of the first instillation of tuberculin.* Second instillations in a few instances elicit reaction in non-tubercular animals.

2. In the majority of animals tested the reaction increased in its intensity with each subsequent instillation of tuberculin. This fact indicates the development of a local hypersusceptibility or anaphylaxis associated with a partial immunity; von Pirquet calls this condition "*allergie*" (9).

3. It is possible in some cases to create a condition of "*allergie*" in healthy cattle, when spaced instillations of tuberculin are made. It is evident, therefore, that the result of the first instillation of tuberculin should be made the only basis of diagnosis. Rosenau and Anderson (11) have recently called attention to this point in regard to the human subject.

4. When repeated instillations of tuberculin are made on the conjunctiva at short intervals (twenty-four hours, etc.) a local immunity results (No. 3D *et al.*). If the instillations are separated two weeks or more anaphylaxis results.

5. We, therefore, hold that if tuberculin (0.1 cubic centimeter) is carefully instilled into the conjunctival sac and if careful comparison of the instilled eye with the opposite eye shows that a reaction of varying intensity results in from ten to twelve hours after the first instillation, a tubercular lesion is present.

6. In our first report (7) we were inclined to believe that subcutaneous tubercular injection given previous to the ocular test would slightly inhibit it. We have since become convinced that this

NECROPSY REPORT.

Class A.

	Retropharyngeal Glands.	Mesenteric Glands.	Portal Glands.	Liver.	Peritoneum.	Uterus.	Lungs.	Bronchial Glands.	Posterior Mediastinal Glands.	Udder.	Pleura.	Glands of Muscles.	Bones.	Kidney.	Intestine.
1 *A*	o	o	o	o	o	o	o	x +	o	o	o	o	o	o	o
2 *A*	o	o	o	xx +	o	o	xx +	o	xxx +	o	o	o	o	o	o
3 *A*	x +	o	o	x +	o	o	xx +2	x +	xxx +	xxx +B	xxx +	o	o	o	o
4 *A*	o	o	o	o	o	x +	xx +2	x +	xxx +	o	o	x +	o	o	o
5 *A*	o	o	o	x +		+	xxx +2	xx +	x +	o	o	o	o	o	o

Class B.

	Retropharyngeal Glands.	Mesenteric Glands.	Portal Glands.	Liver.	Peritoneum.	Uterus.	Lungs.	Bronchial Glands.	Posterior Mediastinal Glands.	Udder.	Pleura.	Glands of Muscles.	Bones.	Kidney.	Intestine.
1 *B*	o	o	o	o	o	o	xx +	o	xx +	o	o	o	o	o	o
2 *B*	x +	xx +	o	o	o	o	o	o	o	o	o	o	o	o	xxxB +L
3 *B*	o	o	o	x +	o	o	xx +	o	xxx +	o	o	o	o	o	o
4 *B*	o	x +	xxx +	x +	o	o	xxx +2	xxx +	xxx +	o	xx +	o	o	o	o
5 *B*	o	o	o	o	o	o	x +2	x +	xxx +	o	o	o	o	o	o

Class C.

	Retropharyngeal Glands.	Mesenteric Glands.	Portal Glands.	Liver.	Peritoneum.	Uterus.	Lungs.	Bronchial Glands.	Posterior Mediastinal Glands.	Udder.	Pleura.	Glands of Muscles.	Bones.	Kidney.	Intestine.
2 *C*	o	o	o	o	o	o	xx +	xx +	xx +	o	o	o	o	o	o
8 *C*	o	o	o	o	o	o	xxx +2	x +	xx +	o	o	o	o	o	o
10 *C*	o	x +	o	o	o	o	xx +	x +	xx +	o	o	o	o	o	o

Class D.

	Retropharyngeal Glands.	Mesenteric Glands.	Portal Glands.	Liver.	Peritoneum.	Uterus.	Lungs.	Bronchial Glands.	Posterior Mediastinal Glands.	Udder.	Pleura.	Glands of Muscles.	Bones.	Kidney.	Intestine.
1 *D*	o	o	o	o	o	o	x +	x +	xx +	o	o	o	o	x +	o
2 *D*	o	x +	o	x +	o	o	o	o	x +	x +B	o	o	o	o	o
3 *D*	o	o	o	o	o	o	xx +2	x +	x +	o	o	o	o	o	o
4 *D*	o	o	o	o	o	o	o	o	x +	o	o	o	o	o	o

NECROPSY REPORT.—*Continued.*

Class D.

	Retropharyngeal Glands.	Mesenteric Glands.	Portal Glands.	Liver.	Peritoneum.	Uterus.	Lungs.	Bronchial Glands.	Posterior Mediastinal Glands.	Udder.	Pleura.	Glands of Muscles.	Bones.	Kidney.	Intestine.
5 *D*	o	o	o	x +	o	o	o	o	xxx +	o	o	o	xxx +	o	o
6 *D*	o	o	o	x +	o	o	x +	o	xxx +	o	o	o	o	o	o
7 *D*	o	o	o	o	o	o	x +	+	xx +	o	o	o	o	o	o
8 *D*	x +	o	o	o	o	o	x +	o	x +	o	o	o	o	o	o
9 *D*	o	o	o	o	o	o	o	o	xx +	o	o	o	o	o	o
10 *D*	o	o	o	o	o	o	o	o	xx +	x +B	o	o	o	o	o
11 *D*	o	o	x +	o	o	o	o	o	xx +	o	o	o	o	o	o
12 *D*	o	xx +	o	xx +	o	o	xx +2	x +	xxx +	o	o	o	o	o	o
13 *D*	x +	o	o	o	o	o	o	o	xx +	x +	o	o	o	x +	o
14 *D*	o	+	o	o	o	o	xx +2	xx +	xx +	o	o	x +	o	o	x +S
15 *D*	o	o	o	o	o	o	xx +	xx +	xxx +	o	o	o	o	o	o

x = Lesions few and small.
xx = Lesions many and small.
xxx = Lesions large.
B = Tubercle bacillus demonstrated.
2 = Both lungs.
o = No lesions demonstrated.
Intestine—S = small intestine; L = large intestine.

is true only to a limited extent, and that in some cases the ophthalmo-reaction is exaggerated by a subcutaneous injection of tuberculin.

7. The primary ophthalmo-tuberculin reaction is in direct proportion to the extent of the tubercular processes in the body. The more extensive the tubercular processes, the more anaphylactic the animal is. This is in direct variance with the condition in the usual subcutaneous tuberculin test. (See Necropsy Report.)

FIG. 1.

8. We are inclined to believe that the ophthalmo-tuberculin test will reveal tuberculosis at as early a state as the usual subcutaneous test.

9. The ophthalmo-reaction is of no value in determining whether vaccinated cattle are actively tubercular or not, or in demonstrating any hypersusceptibility in the offspring of tubercular cattle.

10. The cutaneous test from our brief series of experiments does not seem to be as accurate as the ophthalmic test. This conclusion has been reached by several investigators.

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FURTHER OBSERVATIONS ON ANAPHYLAXIS TO HORSE SERUM.*

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In a previous paper (1) on the reaction of the guinea-pig to single and repeated doses of horse serum, I took the view that the violent reaction obtained at a second treatment which follows the first after a considerable incubation period, was due to the participation in the reaction of a specific reaction product or anti-body developed by the animal during the interval. In support of this position, it was shown that if a considerable quantity of the defibrinated blood of a guinea-pig which had been treated some weeks previously with a mixture of diphtheria toxin and antitoxic horse serum is injected into the peritoneal cavity of a young, normal, untreated guinea-pig the latter becomes hypersensitive to horse serum within twenty-four hours. These experiments were in agreement with those reported by Otto (2) when my paper was being prepared for the press. Moreover, there seemed to be a sharp distinction between this substance which may render the fresh animal hypersensitive within a few hours, and the substance demonstrated by Gay and Southard (3), which gives to a small quantity of the blood serum of an actively sensitized guinea-pig the property of transferring the hypersensitive condition of a " fresh " animal if an incubation period of two weeks be allowed to elapse. In further support of the view that a specific anti-body is developed was the fact first brought out by Rosenau and Anderson (4), that the offspring of a treated female animal are hypersensitive. It was reasoned that in its fundamentals the transmitted hypersensitiveness resembled the more carefully studied cases of transmitted antitoxic immunity. The former seemed further to have in com-

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mon with the latter, a transient character, and a limitation of its effect to the immediate offspring of the treated mother. Recently I have had the opportunity to study more carefully the hypersensitiveness in these young animals born of treated mothers, and it is the results of this study which I wish chiefly to report at present. I have also been able to make some limited observations which have a more practical bearing on the question of serum therapy. The brief report of these will be found in the closing paragraphs of this paper.

The blood of the young guinea-pig hypersensitive by breeding was first subjected to the same tests which had previously been applied to that of animals actively sensitized. Animals of 225 to 250 grams weight were chosen. They were bled, the blood defibrinated and mixed. In the decisive experiments enough animals were used at one time to give a total quantity of twenty cubic centimeters of defibrinated blood. This was at once injected into the peritoneal cavity of fresh guinea-pigs of normal ancestry, weighing from 230 to 250 grams. Two animals were used for each test: one was injected with fifteen cubic centimeters of the blood, the other with from one to five cubic centimeters. The animal receiving the larger quantity was treated with horse serum the next day; that receiving the smaller amount after about twenty days. The test injection consisted in each case of two cubic centimeters of horse serum given directly into the circulation by the intracardiac method. These experiments and the results obtained are described in the following protocols:

Experiment 1.—Guinea-pigs No. 7066, 7067, 7068 were born of treated mother No. 3931. When 3 weeks old the animals were bled. Pooled defibrinated blood, 12 c.c., were injected into peritoneal cavity of guinea-pig No. 7048 (normal, wt. 240 grm.). After 17 hours injected No. 7048 with 2 c.c. serum of Horse 106 by intracardiac method. Dead in 2 minutes. Symptoms: typical. Autopsy: lung and heart hemorrhages.

Experiment 2.—Guinea-pigs No. 7074, 7075, 7076, born of treated mother No. 4875. When 34 days old, bled (25–XI–07). Injection of 5 c.c. mixed defibrinated blood into peritoneal cavity of normal guinea-pig No. 7071. After 30 hours treated No. 7071 with 2 c.c. serum of Horse 106 (intracardiac). Slight but definite symptoms characteristic of serum intoxication. 25–XI–07.—Injection of 1 c.c. of above defibrinated blood into peritoneal cavity of normal guinea-pig No. 7087.

14–XII–07. Treated No. 7087 with 2 c.c. serum of Horse 106 (intracardiac). No symptoms.

Experiment 3.—29–XII–07. Guinea-pigs No. 8035, 8036, 5 weeks old, born of treated mother No. 4877, and guinea-pig No. 7098, 6 weeks old, born of treated mother No. 4876, were bled. Blood was defibrinated and mixed. Injected 15 c.c. of mixed defibrinated blood into peritoneal cavity of normal guinea-pig No. 8003 and 2 c.c. into peritoneal cavity of normal guinea-pig No. 8031.

30–XII–07. Treated guinea-pig No. 8003 received 2 c.c. serum of Horse 109 (intracardiac). The animal was very sick from five minutes to one hour. Recovered. 2–I–08.—Chloroformed. Autopsy shows hemorrhage in lungs and erosion of stomach.

18–I–08. Treated guinea-pig No. 8031 received 2 c.c. serum of Horse 98 (intracardiac). No symptoms whatever.

Experiment 4.—Guinea-pigs No. 8093, 8104, born of treated mother No. 4868, 2½ months old. Guinea-pigs No. 8096, 8105, born of treated mother No. 4865, 2½ months old. Guinea-pig No. 8061, born of treated mother No. 4789, 3 months old.

8–III–08. Above animals were bled; blood was defibrinated and mixed. Injected at once into two young normal guinea-pigs as follows: No. 8150 received 15 c.c. intra-peritoneally. No. 8151 received 15 c.c. intra-peritoneally and 5 c.c. sub-cututaneously.

10–III–08. Treated No. 8150 received 2 c.c. serum of Horse 113 (intracardiac). Slight, but definite symptoms.

27–III–08. Treated No. 8151 received 2 c.c. serum of Horse 113 (intracardiac). No symptoms whatever.

It is shown that the blood of young animals hypersensitive by breeding has the property of rendering a normal animal hypersensitive if the quantity of blood transferred is large, and if the test injection is made within a short time. Thirty-six hours was the longest interval allowed. The degree of hypersensitiveness developed seemed somewhat less for equivalent amounts of blood transferred than that in the cases reported in my earlier paper, where the animals sensitized directly by treatment were used as the source of the blood. In Experiment 1, however, the test injection killed in a few minutes, showing that the immediate sensitiveness developed may under favorable conditions be roughly equal in the two cases. It is of interest that this most intense reaction was developed when using blood from very young animals.

The tests after an incubation period have been entirely and uniformly negative. In Experiment 4 the test of this point was made very severe. While the animal treated after thirty-six hours gave a definite reaction, the one treated after the incubation period gave no reaction whatever, although the amount of defibrinated blood used to sensitize was larger in the latter instance. These experi-

ments are in direct contrast to those performed by Gay and Southard (3), and later by myself, in which it was determined that while, as I have said, with a large amount of the blood of animals actively sensitized it was possible to transfer the hypersensitive condition within a few hours, it was much easier to accomplish this by using a small amount, one tenth to one and a half cubic centimeters, and allowing a period of two weeks to elapse before the test injection was made. Somewhat differently stated, the facts seem to be that in the blood of young guinea-pigs hypersensitive by reason of their breeding, there is a substance which renders the "fresh" animal to which it is transferred hypersensitive within a few hours. In the same blood the anaphylactin of Gay and Southard, which has the property of rendering the animal into which it is injected hypersusceptible after an incubation period, cannot be demonstrated.

Very important in an estimate of the nature of the transmitted hypersensitiveness is the question: Is it a permanent or transitory condition? In my earlier paper I gave report of results which seemed to show that it was transient. Tested by the subcutaneous method when several weeks old, only about fifty per cent. of the animals gave a reaction. It seems best to assume that all of the young bred of a hypersensitive mother acquire a measure of her abnormal condition, and that the observed differences in reaction are due to quantitative differences in the degree of transmission influenced by the rate of loss of the sensitivity. The material has not so far been available for a complete determination of the amount of variation which there may be in the initial intensity of the transmitted reaction. On the probable assumption that any such variations are dominated, in the experiments so far done, by the rate of loss of the abnormal condition, I have been able to make a few observations which more definitely fix the duration of the latter. Of two animals of the same litter, one tested with two cubic centimeters of horse serum (intracardiac), when two and a half months old, died in two minutes; the other subjected to the same test when four months old showed no symptoms. Another animal bred of another mother tested in the same way when four months old gave no reaction. This evidence, though small in amount, is consistent

with the view that the acquired hypersensitiveness is lost within the first few months of the animal's life. It is of especial value in that the test is as severe as it can well be made.

If the conditions in the immediate offspring of treated mothers were identical, except in degree with those in the mother, it might be expected that in favorable instances the grandchildren would be found abnormal. In the earlier records of the laboratory there were found notes on several cases in which horse serum had been given subcutaneously to the grandchildren of treated mothers, the intermediate offspring having been untreated. These animals had never given any reaction, but it was felt that the tests were not perfectly satisfactory, in that the earliest litters of the mothers in question had not been used. I repeated the experiments, using the first offspring of the untreated mother at an early age, but got no reaction. On this basis, I felt justified in using the argument that transmitted hypersensitiveness did not occur beyond the first generation. Since becoming familiar with the great delicacy of the reaction when the test injection is made directly into the circulation, it has seemed worth while to reexamine this question. Eight guinea-pigs born of five different untreated mothers, descendants of five treated grandmothers, were tested. Together with these were used five control animals from a source which made it certain that none of the ancestors for several generations could have been treated in any way with horse serum. The animals were used at weights varying between 175 grams and 250 grams. The least severe test applied was the injection of two cubic centimeters of horse serum by the intracardiac method. In several instances, additional serum was administered within a few minutes into the peritoneal cavity, or subcutaneous tissue, or both. As much as ten cubic centimeters were given in several instances over and above the intracirculatory dose. The result was conclusive in showing that there is no hypersensitiveness transmitted beyond the immediate offspring.

These experiments have also been instructive in showing the limit to which horse serum can be given to normal guinea-pigs without producing symptoms of serum poisoning. In no instance where the injection was limited to two cubic centimeters into the

circulation were there symptoms produced.* When. however, this was followed by five centimeters given into the peritoneal cavity in each case there resulted a very slight reaction more or less characteristic of the earliest stage of the hypersensitive reaction. For half an hour after this maximum treatment the animals have shown short periods of uneasiness, alternating with periods of unusual quietness. They shiver somewhat, show twitching and slight involuntary convulsive movements of the limbs or diaphragm. Occasionally, they sneeze or rub the ears and nose. These symptoms pass away in a short time. In several instances where they have been most definite, the animals have been killed after a day or two. Examination, except in one case, has revealed no lesion whatever. In this case there were irregular subserous hemorrhages in the peritoneal cavity, slight swelling of one or two mesenteric lymph nodes, with some hemorrhage into their sinuses, and a few hemorrhages in the lungs. These lesions are in accord with those found in the hypersensitive animal during the phase of intoxication. Of course, not too much stress should be laid on this single case. The experience with these young guinea-pigs treated with large doses of serum is, it would seem, most consistent with the view that horse serum is not a perfectly indifferent substance for this animal, but is in reality a mild poison. The intoxication obtained in the hypersensitive animal in the light of these results is not an adventitious reaction, but is an exaggeration of a reaction which occurs when the normal animal is treated with horse serum.

It may be profitable to discuss briefly the possible manner of the hypersensitive reaction of the guinea-pig to horse serum from the point of view of the experiments with the animals of abnormal breeding. As before stated, I have held to the view that the sensitizing treatment causes a reaction on the part of the animal, in the course of which an anti-body is developed in excess. This

* In the course of these tests it was found that when so large an amount of serum as this is injected into the circulation, if chloroform has been used as a preservative it must be exhausted before use. Otherwise, there is instantly produced a profound general anæsthesia, which may result in death, although it usually passes off in a few minutes. A serum which has no taste of chloroform can be used with safety.

anti-body meeting with the second injection very greatly accelerates a reaction which at one or another stage is injurious to the cells of the animal. In holding this conception of the mechanism of the reaction of anaphylaxis, I am neither alone nor original. The same idea was first expressed by v. Perquet and Shick (5), and later by Currie (6), to explain the phenomena of the serum disease in human beings. They derived the idea from theoretical considerations, obviously under the influence of current theories of immunity reactions. Nicolle (7) holds this view with regard to the reaction of the rabbit to horse serum. Otto (2) expressed the same opinion as to the process in the guinea-pig, and Richet (8) has recently indicated a similar mechanism for the reaction of the dog to mytilo-congestin. The three latter observers brought to bear experimental evidence for their opinion, in that they showed that in each instance it was possible to render a fresh animal hypersensitive within twenty-four hours, by treating it with the blood or blood serum of a hypersensitive animal.

Nor can the following partial conclusion quoted from the paper of Gay and Southard be considered as fundamentally inconsistent with this idea: "The reaction of intoxication would seem to be a cellular one, dependent upon a heightened power of assimilation on the part of cells which have been subjected to the anaphylactic substance over a definite period of incubation." But further than this, they hold that the sensitizing and toxic actions of the horse serum are dependent on two distinct substances. On this assumption, the incubation period might be occupied with the elimination of the assimilable toxic portion, and at its completion the sensitizing, non-toxic portion, the anaphylactin in their terms, which is eliminated but slowly, would be isolated or left uncovered. The serum of the hypersensitive animal would then contain purified anaphylactin. Now, it could perhaps be argued that the purified sensitizing substance could act efficiently in developing a given degree of hypersusceptibility in a shorter or longer time, depending directly on the quantity present. In sensitizing an animal with the blood of a hypersensitive animal according to this view, when one-tenth of a cubic centimeter sensitizes in two weeks, and fifteen cubic centimeters in twenty-four hours, the inference to be drawn is not that

there are two distinct active substances in the blood used, as I have supposed, but one substance, the anaphylactin, which acts quickly in large amount, but continuously and, in the end, effectively in the smaller amount. It is not necessary at present to dwell on the indications found within the series of experiments with the blood of serum-treated animals that tended to distinguish two active substances. However plausible such an interpretation as outlined may appear to be for those animals actively sensitized, it is not at all in harmony with the experiments on the blood of the animals hypersensitive by breeding. If the sensitization of normal animals with this blood were due to the direct action of anaphylactin, the animals tested after two weeks should have been found fully as sensitive, and perhaps more so, than the animals tested at twenty-four hours, or thereabout. Guinea-pig No. 8151 of Experiment 4 should have been more sensitive than its fellow, No. 8150. The reverse was true.

On the strength of the presumably transitory character of the hypersensitive condition in the cases of acquired hypersusceptibility, the limitation of the condition to the immediate offspring of the treated mother, and especially because of the impossibility of demonstrating the sensitizing horse serum element in the blood of these animals while it is possible to transfer the condition passively to a " fresh " animal by the use of their blood, the conclusion that the acute reaction to the intoxicating injection is due to the participation in the reaction of a newly formed substance or antibody would seem justifiable. To those who have had the patience to follow the experiments and argument thus far, it will be sufficiently obvious that to have reached such a conclusion is but to have made one step toward the understanding of a most complex problem.

In closing, I wish to discuss very briefly some of the more practical questions that have arisen in connection with the study of the serum reaction in the guinea-pig, other lower animals, and man. Influenced by his work on the reaction in the guinea-pig, Besredka (9) has proposed that all antitoxic sera should be tested on the hypersensitive animal to be sure that they have no unusual toxicity in this reaction before they are marketed. In the light of a proposition of such practical importance, the following observation is of

interest. During the past winter, a sample of antitoxin was referred back to the laboratory for examination. A prophylactic injection of about three cubic centimeters of the serum had caused very severe œdema of the face, pharynx, and larynx, in a healthy adult male. The effect came on within half an hour after the injection, lasted for some time, and at its height, the symptoms were very alarming. The fact that this patient's wife, who received an injection of about nine cubic centimeters of the same lot of serum, experienced no reaction, would almost be a sufficient indication that this untoward result was not due to any unusual quality in the serum. Tested on the hypersensitive guinea-pig, this serum showed a toxicity not materially different from the various normal sera which I have used in the course of this work. One two-hundredth of a cubic centimeter caused severe symptoms with recovery, when injected directly into the circulation. One one-hundredth cubic centimeter is probably a certainly fatal dose of normal serum under the conditions of this test, so that this lot of serum could not have had a toxicity of twice the normal, supposing it to have been at all above the average.

During the past winter some experiments have been undertaken on the reaction of the rabbit to horse serum. It is probable from the work of Arthus, Nicolle, and others, that this animal reacts fundamentally in the same way as does the guinea-pig. Certain distinctions become apparent at once, however. It is much more difficult to render the rabbit hypersensitive to such a degree that the intravenous injection of serum will kill the animal. The single treatment with a mixture of diphtheria toxin and antitoxin, which is so efficient in rendering the guinea-pig hypersensitive, is without any demonstrable effect on the rabbit. While the underlying principles of the reaction are probably the same in the two animals, the factors in each case are so different in their relative values that a treatment which is certainly fatal for the guinea-pig has no appreciable effect on the health of the rabbit. In attempting to determine whether a given serum treatment is or is not dangerous, evidently each species of animal must be separately considered. It is almost needless to point out that the data accumulated since 1893 on the accidents incident to the therapeutic use of horse serum, its

uncomfortable sequelæ, and its great benefits are of much more value, as a guide for future practice, than conclusions drawn from complex experiments on the laboratory animals. It would be a most unfortunate presentation of laboratory results on anaphylaxis which should lend itself to even a temporary or slight reaction against a therapy which has so thoroughly justified itself in the case of some diseases, and which offers such possibilities for the future in the case of others.

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A STUDY OF THE PROTEOLYTIC FERMENTS OF THE LARGE LYMPHOCYTES IN A CASE OF ACUTE LEUKÆMIA.*

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At least thirty years ago chemical investigations made upon the blood of persons suffering from leukæmia seemed to show that in those forms of the disease characterized by the presence of granular myelocytes peptone was formed in the blood. Schumm,[1] in 1903, pointed out that peptones were only formed in the blood after it had been shed, while Erben,[2] at the same time, demonstrated that the leucocytes from the blood of cases of myelogenous leukæmia contained a ferment which was capable of digesting fibrin. Digestion took place best if the fibrin and cells were suspended in an alkaline fluid, though some evidence of the proteolysis could be seen when acid was used. That both the polymorphonuclear leucocytes and the neutrophilic granular myelocytes contain enzymes that are capable of digesting blood serum at 55° or 60° C. or gelatin at 36°C. has been amply demonstrated by Jochmann and Müller[3] and Stern and Eppenstein.[4] These investigators have found repeatedly this enzymotic action in the cells from the blood of cases of myelogenous leukæmia, but have failed to demonstrate that the

* Received for publication June 12, 1908.

[1] Schumm, *Beit. zur chem. Physiol. u. Path.*, 1903, iv, 442.

[2] Erben, *Zeit. f. Heilk.*, 1903, xxiv, 70. *Beit. zur chem. Physiol. u. Path.*, 1904, v, 461.

[3] Jochmann and Müller, *Münch. med. Woch.*, 1906, liii, 1393, 1507, 2002, 2093.

[4] Eppenstein, *Münch. med. Woch.*, 1906, liii, 2192; Stern and Eppenstein, *Allg. med. central Zeit.*, 1906, lxxv, 552.

lymphocytes either from cases of lymphatic leukæmia or the lymph nodes from cases of pseudo-leukæmia possess any such action.

Eppenstein[5] was unable to show any biological differences between the large lymphocytes of the blood from a case of acute lymphatic leukæmia and the small lymphocytes from cases of chronic lymphatic leukæmia. Luksch[6] found in two cases of myelogenous leukæmia, both of which had very high leucocyte counts, that a drop of blood produced cupping of the surface of coagulated blood serum at 55° C. Blood from a case of what he terms lymphatic leukæmia, showing a leucocyte count of 8,600, 51 per cent. of which were mononuclears and 49 per cent. polynuclears, gave no digestion. The diagnosis in a second case was somewhat doubtful, for there was no study of the blood, but the lymph nodes were enlarged and contained large lymphocytes and there were nodules in the spleen. The cells of the bone marrow and the spleen contained proteolytic ferments, but the lymph glands were without enzymotic action. In a third case the leucocyte count was 180,000. Of the white cells 98 per cent. were non-granular cells half of them small lymphocytes and the other half large, while all degrees of transition between the two were observed. The blood from this case did not digest the blood serum. Luksch's conclusions, that the large lymphocytes in cases of lymphatic leukæmia are without ferments and act, therefore, like the small lymphocytes, do not seem to be entirely justified by his experiments, for in the first case there were probably not sufficient white cells in the drop of blood, otherwise one would expect to see some evidence of action from the polymorphonuclear leucocytes, which are known to contain ferments and which were present in approximately the same numbers as the lymphocytes. The diagnosis in the second case is too uncertain to enable one to draw conclusions, while the third case was evidently not one of the pure large cell type of leukæmia. Müller[7] states that in one case of acute lymphatic leukæmia the cells acted upon coagulated blood serum at 55°–60° C. in the same manner as polymorphonuclear leucocytes, digesting the proteid when the reaction was alkaline or neutral.

[5] Eppenstein, *Deutch. med. Woch.*, 1907, xxxiii, 1984.
[6] Luksch, *Folia haemat.*, 1908, v, 75.
[7] Müller, *Deutsch. Arch. f. klin. Med.*, 1907, xci, 291.

Opie[8] has demonstrated the importance of the reaction of the fluid in which the leucocytes are allowed to act. He has observed that the cells obtained from pleurisies produced experimentally in dogs show two types of digestion, corresponding to the two varieties of cells found in the exudates. The enzymes derived from the polymorphonuclear leucocytes or microphages act best in an alkaline or neutral solution, whereas the other enzymes derived from the large phagocytic cells or macrophages act almost exclusively in an acid reaction. In neutral or alkaline solutions Opie found that emulsions of lymph glands from dogs, rich in these large phagocytic cells, have practically no proteolytic action, while in dilute acid suspension the emulsions showed definite enzymotic activity.

In the presence of fresh unheated blood serum, the leucocytes and large phagocytes are practically inactive, for the blood serum, as has been demonstrated by Opie,[9] Jochmann and Müller,[10] Wiens,[11] Bittorf,[12] and Wiens and Müller,[13] contains an anti-ferment which though injured when heated to 55°–60° C. is not entirely destroyed until the serum is heated to 75° C. for several minutes.

In view of the discussion as to the origin of the large lymphocytes in acute lymphatic leukæmia and their relationship to the granular myelocytes on the one hand and the non-granular lymphocytes on the other, it seemed of importance to establish, if possible, the position of these cells on a biological basis.

Since it was necessary to study the leucocytes of the circulating blood, a method had to be devised for obtaining the white cells as free as possible from the admixture of the red corpuscles. It was, moreover, important to free the white cells from the serum, on account of the property which the blood serum has of inhibiting the enzymotic action of the leucocytes. To procure the lympho-

[8] Opie, *Jour. of Exper. Med.*, 1905, vii, 316; 1906, viii, 410; 1907, ix, 391 and 414.

[9] Opie, *Jour. of Exper. Med.*, 1905, vii, 316; Opie and Barker, *ibid.*, 1907, ix, 207.

[10] *Loc. cit.*

[11] Wiens, *Münch. med. Woch.*, 1907, liv, 3637; *Deutch. Arch. f. klin. Med.*, 1907, xci, 456.

[12] Bittorf, *Deutsch. Arch. f. klin. Med.*, 1907, cxi, 212.

[13] Wiens and Müller, *Zent. f. inner. Med.*, 1907, xxviii, 945.

cytes in as great a concentration as possible the blood, drawn from the arm vein, was diluted with several times its volume of 1.5 per cent. sodium citrate, the mixture centrifugalized and the upper buffy layer withdrawn. As could be determined by microscopical examination, this layer contained practically all the leucocytes. The mixture of leucocytes and red blood cells was then washed three or four times in 0.85 per cent. sodium chloride suspended in a given quantity of this solution and used for the experiments. In a few instances one per cent. ammonium oxalate was employed instead of sodium citrate. As the ammonium oxalate destroyed the red blood cells the leucocytes were obtained in pure state at the bottom of the centrifuge tube.

In the first experiments only qualitative determinations of the proteolytic action of the leucocytes were made and for this purpose the method described by Jochmann and Müller was adopted. A mixture of horse serum and bouillon, which was approximately the same as is recommended for the preparation of Löfflers' blood serum, was coagulated in Petri dishes. Upon these plates were dropped equal quantities of the suspension of leucocytes and a 0.85 per cent. sodium chloride solution, 0.1 and 0.2 per cent. sodium carbonate and 0.1 and 0.2 per cent. hydrochloric acid. One set of plates was usually incubated at 37° C., another set at 50–55° C. Jochmann and Müller state that proteolysis is much more active at the latter temperature than at 37° C. The presence of proteolysis was estimated by cupping and liquefaction of that portion of the blood serum covered by the drop of leucocytic suspension.

By this method it could be shown that the leucocytes of the blood of normal individuals contained definite enzymes, which acted in neutral, akaline, and acid media and seemed to digest best at 55° C.

XIV. Healthy individual. January 10, 1908.

Twenty c.c. of blood were withdrawn and put into 30 c.c. 1 per cent. ammonium oxalate. Centrifugalized sediment was washed three times in 0.85 per cent. sodium chloride. Sediment consists exclusively of leucocytes, principally of the polymorphonuclear variety.

	37° C. 20 hrs.	37° C. 44 hrs	55° C. 20 hrs.	55° C 44 hrs
Equal parts sed. + 0.85 per cent. sod. chloride	+ —	+	+	+
Equal parts sed. + 0.1 per cent. sod. carbonate	+ —	+	+	+
Equal parts sed. + 0.1 per cent. hydrochloric ac.	—	—	+ +	+ +
Equal parts sed. + 0.2 per cent. hydrochloric ac.	+	+	+ +	+ +

— = No cupping of coagulated serum.
+ — = Uncertain cupping of coagulated serum.
+ = Definite cupping of coagulated serum.
+ + = Deep cupping of coagulated serum.

The leucocytic sediments from cases in which the leucocyte count showed a marked increase of the white cells acted in much the same manner, except that digestion was often more marked.

IV. December 15, 1907. Pneumonia.

Twenty c.c. of blood put into 15 c.c. ammonium oxalate; 0.5 per cent. white sediment, consisting entirely of leucocytes, was washed three times in 0.85 per cent. sod. chloride and made up to 2 c.c. with the same solution.

	37° C. 16 hrs.	37° C. 40 hrs.	55° C. 16 hrs.	55° C. 40 hrs.
0.1 c.c. sed. + 0.1 c.c. 0.85 per cent. sod. chloride	+—	++	+++	++++
0.1 c.c. sed. + 0.1 c.c. 0.1 per cent. sod. carbonate	+—	++	+++	++++
0.1 c.c. sed. + 0.1 c.c. 0.1 per cent. hydrochloric ac.	+—	++	+++	++++

+++ = cupping with liquefaction.

Washed pus was found to act in exactly the same manner as the leucocytes from the blood of normal individuals and of persons showing a marked leucocytosis. As it was impossible to obtain pus which was sterile, specimens were chosen which contained either pneumococci or streptococci, organisms that have little or no proteolytic action.

VII. Thick white pus contains pneumococci in pure culture.

Microscopical examination shows that the cells are almost excusively polymorphonuclear leucocytes; very rarely a mononuclear cell is seen.

Pus washed three times in 0.85 per cent. sodium chloride, used without dilution.

	37° C.		55° C.	
	42 hrs.	66 hrs.	42 hrs.	66 hrs.
Equal parts pus + 0.85 per cent. sod. chloride	+	+++	+++	++++
Equal parts pus + 0.1 per cent. sod. carbonate	+	+++	+++	++++
Equal parts pus + 0.1 per cent. hydrochloric ac.	+	+++	+++	++++

This simple method, which we first adopted, gave very striking evidence of the existence of proteolytic activity of the leucocytes, but afforded no definite information by which we could compare the action obtained in one experiment with that observed in another. It was therefore decided to estimate accurately the amount of digestion by the method of determining the nitrogen of the uncoagulable proteid, as suggested by Opie. For this purpose, measured quantities of the leucocytic sediments were allowed to act on measured quantities of heated blood serum in neutral, alkaline and acid sus-

pensions. Horse serum or human serum was diluted with three parts of 0.85 per cent. sodium chloride and heated for half an hour at 75°–80° C. When the serum is thus diluted, heating produces no coagulum, but a thin milky fluid which can readily be measured in a pipette. Measured quantities of the suspension of cells to be tested were added to 10 cubic centimeters of the heated serum in a flask with a capacity of 200 cubic centimeters and the total quantity made up to 20 cubic centimeters by the addition of 0.85 sodium chloride, sodium carbonate or acetic acid. The addition of sodium carbonate and acetic acid was so arranged that the resulting mixture would be 0.2 per cent. alkaline or 0.2 per cent. acid.

To each flask one cubic centimeter of toluol was then added. Finally the flasks were stopped with corks and placed in the thermostat, usually for five days. As a control the suspension of cells was boiled and treated in the same manner as described. Boiling for two or three minutes was found to destroy completely the proteolytic action of the cells.

Most of these experiments were conducted at 36°–37° C. At the end of five days, after the addition of equal quantities of 16 per cent. magnesium sulphate, the contents of the flasks were boiled, acidified with dilute acetic acid, boiled again, neutralized and filtered directly into Kjeldahl flasks. The small flasks were washed several times, the washings poured onto the filter and the coagulum in the filter finally washed again. The nitrogen of the filtrate was determined by the Kjeldahl method, the quantity of nitrogen in uncoagulable form representing the amount of proteid digested. The figures in the tables represent the number of cubic centimeters of N/10 hydrochloric acid neutralized and not the total nitrogen. All the experiments were performed in duplicate.

By this method it was found, as Opie has already stated, that the polymorphonuclear leucocytes digested best in an alkaline or neutral medium and very slightly in an acid medium. The following experiment demonstrates this fact definitely.

XXVI. March 18. Thick, yellow pus from ankle is composed almost exclusively of polymorphonuclear leucocytes. Cultures give Streptococcus pyogenes. Pus is washed three times in 0.85 per cent. sodium chloride. 3 c.c. washed cells are suspended in 9 c.c. 0.85 per cent. sodium chloride. 1 c.c. of this mixture = .33 c.c. cells.

1 c.c. + horse serum—neutral	16.1
1 c.c. pus + horse serum—0.2 per cent. alkaline	18.4
1 c.c. pus + horse serum—0.2 per cent. acid	6.3
1 c.c. pus boiled + horse serum—neutral (control)	2.8

5 days at 36.5° C.

Since the main object of these experiments was to study the proteolytic enzymes in the leucocytes in leukæmia, the foregoing protocols may be considered as controls with which it is possible to compare the results of the experiments which follow. It has been shown that the leucocytes of normal blood, of the blood from cases showing a leucocytosis, and of pus, all contain a ferment which has the power of digesting proteids in neutral, alkaline and acid media; and with pus, which is composed chiefly of polymorphonuclear leucocytes, this action takes place best in an alkaline or neutral solution.

It was possible to study the ferments in the leucocytes from the blood of a case of myelogenous leukæmia and it was found that the leucocytes in this case acted in much the same manner as the leucocytes of pus.

The patient was a man under the care of Dr. DaCosta at the Jefferson Hospital. To Dr. DaCosta we are greatly indebted for the opportunity of obtaining the material for study. The patient had suffered from myelogenous leukæmia for years and at the time that the blood was obtained, had an enormous spleen filling most of the abdomen. The leucocytes were 274,000. A differential count showed the following proportion of cells:

		Per Cent.
Polymorphonuclear neutrophiles	330	67.8
Neutrophilic myelocytes	109	21.8
Basophiles	12	2.4
Polymorphonuclear eosinophiles	1	0.2
Eosinophilic myelocytes	3	0.6
Small mononuclears	9	1.8
Large mononuclears	12	2.4
Unidentified cells	15	3.9

It will thus be seen that 89.6 per cent. of the cells were neutrophilic leucocytes and myelocytes.

XXVII. March 27. 15 c.c. of blood in 100 c.c. of 1.5 per cent. sodium citrate is centrifugalized; upper layer is drawn off and is 2.5 c.c., composed

principally of white cells. They are washed four times in 0.85 per cent. sodium chloride made up to 8 c.c. with the same solution. 1 c.c. of the mixture equals .31 c.c. cells.

1 c.c. cell suspension + coagulated horse serum—neutral 19.5
1 c.c. cell suspension + coagulated horse serum—.2 per cent. alkaline 20.5
1 c.c. cell suspension + coagulated horse serum—.2 per cent. acid 13.8
1 c.c. cell suspension boiled + coagulated horse serum—neutral (control). 2.4
5 days at 37° C.

There is perhaps more digestion in acid medium than was obtained with pus alone.

During this study we were most fortunate in having at the Pennsylvania Hospital, under the care of Dr. J. A. Scott, a typical case of acute lymphatic leukæmia. The patient was a young man, a tailor by trade, and twenty-two years of age. When he was admitted on January 19, 1908, he had been acutely ill for five days with fever, headache and abdominal pain, though for two or three weeks he had been feeling unwell and had suffered with what he supposed was "grippe." On admission he showed some pallor, and had slight enlargement of the cervical and inguinal lymph nodes. There was a systolic murmur at the apex of the heart. The liver and spleen were not palpable. The illness progressed rapidly and was characterized by fever, varying from 101° to 104.4°, progressive anæmia, a petechial eruption in the axillæ, repeated epistaxes, vomiting and progressive weakness.

The examination of the blood showed a progressive anæmia with an increasing leucocytosis. The following table shows the actual numbers and percentages of cells.

Differential Counts.

Jan.	Hb.	Rbc.	Lcts.	Poly.	Neut. Myel.	L. Mono.	S. Mono.	Eosin.	Baso.	Unident.
20	39		88,000							
22	39	2,104,000	104,000	16.6	20.2	52.0	5.0	0.2	2.8	3.2
24			120,000							
26	39		114,000							
28	35	1,320,000	170,000							
29	20	1,124,000	238,000	4.7	3.8	76.7	5.0		0.6	9.1
30	17	832,000	414,000	4.1	4.6	77.7	6.7		1.4	5.5
		12 nucleated r.b.c.								

The blood picture was characterized by the presence of typical large lymphocytes. These cells possessed large round, oval, in-

dented or somewhat irregular nuclei, staining rather palely by Wright's stain, which were surrounded by a wide rim of protoplasm taking a faint basophilic stain. Smears stained in Ehrlich's mixture showed no granules in the protoplasm of most of these cells, though a few definite neutrophilic myelocytes were seen.

Since the cells which formed the great bulk of the leucocytes of the blood were thought to be lymphocytes, it was suspected that they might have either no proteolytic action, whatever, or if they did possess a ferment which digested proteid, that this ferment might act differently from the enzymes in the polymorphonuclear leucocytes and granular myelocytes.

The first experiment showed that the cells contained definitely a proteolytic ferment.

XIX. January 27. Leucocytes 350,000. 10 c.c. of blood are drawn in 150 c.c. of 1.5 per cent. sodium citrate. 2 c.c. of sediment which is a mixture of red blood corpuscles and leucocytes are obtained. Sediment is made up to 20 c.c. with 0.85 per cent. sodium chloride; 5 c.c. contains 0.5 c.c. cells.

5 c.c. sediment + human serum—neutral	12.7
5 c.c. sediment boiled + human serum—neutral; control	1.3

5 days at 37° C.

That this proteolytic action is really dependent upon the large lymphocytes and not upon the presence of the small percentage of granular leucocytes, could be definitely shown in later experiments where it was found that the lymph nodes from this case, which did not contain any granular cells, acted in the same way as the cells of the blood.

The case terminated fatally and immediately after death a trocar was inserted into the right side of the heart and blood withdrawn under aseptic conditions into 1.5 per cent. sodium citrate. In this way about 750 cubic centimeters of blood were obtained. The mixture, which looked like yellow pus with a thin layer of blood at the bottom, was kept at 5° C. By centrifugalization pure leucocytes, free from red blood cells could be obtained.

The following experiment shows that these cells digest best in alkaline and neutral media, but are also quite active in the presence of acid.

XX. Twelve c.c. of washed leucocytes are made up to 60 c.c. with 0.85 per cent. sodium chloride. 5 c.c. of this mixture equal 1 c.c. of leucocytes.

5 c.c. cells + human serum—neutral 16.3
5 c.c. cells + human serum—.2 per cent. alkaline..... 15.9
5 c.c. cells + human serum—.2 per cent. acid..... 11.2
5 c.c. cells boiled + human serum—neutral (control)..... 3.6
5 days at 37° C.

If we compare the figures in this experiment with those obtained by the action of pus and of the leucocytes from the case of myelogenous leukæmia, it may be seen that though the quantity of cells from the case of lymphatic leukæmia was three times as great as that in the other two experiments, digestion was not quite as good in neutral and alkaline media, but somewhat better comparatively in acid medium. Since it could be shown, as will be seen later, that the amount of digestion varied with the quantity of cells employed, it may be suggested that though these large lymphocytes in lymphatic leukæmia possess an enzyme which digests proteid in neutral and alkaline media, the ferment is not present in such great amounts as it is in the myelocytes and neutrophilic polymorphonuclear leucocytes.

The enzyme of the large lymphocytes seems to act better than the enzyme of the myelocytes and polymorphonuclear leucocytes when the reaction of the solution is acid.

Opie found that a powder made from the polymorphonuclear leucocytes retains for a long time that ferment of the fresh cells which digests in neutral and alkaline media, whereas the enzyme acting in acid media is not so stable and in the powder diminishes or disappears.

From the washed leucocytes obtained from this case of lymphatic leukæmia, a powder was prepared in the following manner. The mass of leucocytes was covered first with several times its volume of absolute alcohol. This was repeated two or three times. Next the gummy mass was treated in the same manner with ether, pressed between pads of filter paper, dried at room temperature and finally powdered in a mortar. The freshly prepared powder, in amounts of 50 mgr., appeared to act in much the same way as the fresh, washed leucocytes.

XXII.
50 mgr. powder + horse serum 17.7
50 mgr. powder + horse serum—alkaline 16.5

50 mgr. powder + horse serum—acid	8.8
50 mgr. powder boiled (control)	3.2
50 mgr. powder in 0.85 per cent. sodium chloride	3.2

5 days at 37° C.
The powder itself shows very little autolysis.

After the powder had been kept at room temperature for nineteen days the same quantity as was used in Experiment XXII showed a distinct decrease in enzymotic activity.

XXIV.

50 mgr. powder + horse serum	5.1
50 mgr. powder + horse serum—alkaline	10.9
50 mgr. powder + horse serum—acid	6.4
50 mgr. powder boiled (control)	3.1

5 days at 37° C.

This experiment was repeated several times, always with the same result.

By increasing the quantity of powder to 100 grm. the amount of digestion, when the experiment was done in the presence of 0.85 per cent. sodium chloride, was almost doubled and much increased when the solutions were made alkaline.

XXVIII.

50 mgr. powder + horse serum	5.7
100 mgr. powder + horse serum	11.0
50 mgr. powder + horse serum—alkaline	12.2
100 mgr. powder + horse serum—alkaline	18.5
50 mgr. powder boiled + horse serum (control)	3.8

5 days at 37° C.

From these experiments it may be seen that the white cells in the blood in this case of so-called acute lymphatic leukæmia possess a proteolytic enzyme which, though probably present in the cells in not so great amount, is still qualitatively the same as the proteolytic enzyme of the polymorphonuclear leucocytes and granular myelocytes.

Since the lymph nodes, in this case, were enlarged and on microscopical examination were found to be made up almost exclusively of large cells similar to those found in the circulating blood, it was of interest to study the digestive ferments in these glands and to compare their action with that of normal lymph glands or of lymph glands from other pathological conditions.

As it was possible to obtain only one or two glands, there was not sufficient material to study the enzymotic activity quantitatively, but the following experiments show that the cells of these enlarged glands contained proteolytic enzymes acting best in an alkaline medium.

XXI. Emulsions of inguinal lymph glands are washed three times in 0.85 per cent. sodium chloride.

	20 hrs.	48 hrs.
Emulsion + 0.85 per cent. sod. chloride on coag. serum plate	?	++
Emulsion + 0.2 per cent. sod. carbonate on coag. serum plate	++	+++++
Emulsion + 0.2 per cent. hydrochloric ac. on coag. serum plate	?	++

Plates kept at 55° C.

Lymph glands which showed slight hyperplasia were found to act on coagulated blood serum in an entirely different manner. Enlarged inguinal glands were obtained from a case of carcinoma of the penis. Microscopically the glands showed well-marked lymphoid hyperplasia with some proliferation of the large endothelioid cells.

XV. Emulsions of glands are washed three times in 0.85 per cent. sodium chloride.

	18 hrs.	42 hrs.	66 hrs.
Equal parts of emulsion + 0.85 per cent. sod. chloride	—	—	—
Equal parts of emulsion + 0.1 per cent. sod. carbonate	—	—	—
Equal parts of emulsion + 0.1 per cent. hydrochloric ac.	—	+	++
Equal parts of emulsion + 0.2 per cent. hydrochloric ac.	+—	++	++

Plates kept at 55° C.

It may be seen that the results of this experiment differ absolutely from those obtained from the glands from the case of lymphatic leukæmia, since in the last experiment digestion has taken place only in the presence of acid.

Much the same result was obtained with an emulsion of the hyperplastic mesenteric lymph nodes removed at autopsy from a case of typhoid fever. Since the mesenteric lymph nodes in typhoid fever contain great numbers of large phagocytic cells, described by Mallory and others as endothelioid cells, these lymph nodes seemed to offer excellent material for the comparison of the action of such cells with the large lymphocytes in lymphatic leukæmia. The following experiment demonstrates, however, that emulsions of glands containing great numbers of these large endothelioid cells act en-

tirely differently from the large lymphocytes in the case of leukæmia and digest, as Opie has already shown, almost exclusively in the presence of acid.

XXXII. Thick emulsion of mesenteric lymph nodes in 0.85 per cent. has been obtained from a case of typhoid fever. 2 c.c. of emulsion equal about 0.5 c.c. pure cells.

2 c.c. emulsion + horse serum	4.05
2 c.c. emulsion + horse serum—alkaline	4.0
2 c.c. emulsion + horse serum—acid	12.3
2 c.c. emulsion boiled + horse serum (control)	2.6
2 c.c. emulsion + 0.85 per cent. sod. chloride (control)	3.0

5 days at 37° C.

Finally it was possible to study the action of the glands from a case of chronic lymphatic leukæmia. For the material from this case of leukæmia we are indebted to Dr. Funke and to Dr. William Pepper. The patient, during life, had an exceedingly high leucocyte count, the total number of white cells reaching, at one time, one million. Over 9 per cent. of the cells were small lymphocytes. At autopsy the lymph nodes were enormously enlarged and on section showed a reticular mesh work, crowded almost exclusively with small lymphocytes, similar to those in the blood.

The following experiments show that emulsions of the glands, as well as pieces of the glands themselves, failed to digest coagulated blood serum in any medium.

	24 hrs.	48 hrs.
Emulsion + 0.85 per cent. sod. chloride	—	—
Emulsion + 0.1 per cent. sod. carbonate	—	—
Emulsion + 0.1 per cent. hydrochloric acid	—	—
Pieces of gland + 0.85 per cent. sod. choride	—	—
Pieces of gland + 0.1 per cent. sod. carbonate	—	—
Pieces of gland + 0.1 per cent. hydrochloric acid	—	—

Plates kept at 55° C.

Though it was impossible to obtain lymphocytes of the circulating blood from a case of small cell lymphatic leukæmia, yet the last experiment seems to confirm very definitely the work of Jochmann and Müller, Stern and Eppenstein and others, and shows that the small lymphocytes in the enlarged lymph nodes in that type of lymphatic leukæmia, which is characterized by the presence of small lymphocytes in the blood stream, do not have any proteolytic en-

zyme which is demonstrable by allowing the cells to come in contact with coagulated blood serum in the presence of neutral, alkaline or acid solutions.

CONCLUSIONS.

The leucocytes of the blood of normal individuals and of patients showing a marked polymorphonuclear leucocytosis contain enzymes capable of digesting coagulated blood serum in neutral, alkaline or acid solutions.

The cells in pus that is composed principally of polymorphonuclear leucocytes and the leucocytes of the circulating blood in myelogeneous leukæmia contain similar proteolytic enzymes, which act best when the reaction is alkaline.

The leucocytes of the circulating blood and of the enlarged lymph nodes from a case of large cell, acute, lymphatic leukæmia contain proteolytic enzymes that act qualitatively in much the same way as the leucocytes of pus and as the white corpuscles of the blood in myelogenous leukæmia.

These large lymphocytes in acute lymphatic leukæmia can be differentiated biologically from the small lymphocytes in chronic lymphatic leukæmia which possess no proteolytic enzymes, and from the large endothelioid cells of the hyperplastic lymph glands which are proteolytic only in the presence of acid.

These results seem to show that the large cells of the so-called acute lymphatic leukæmia are not true lymphocytes, but are nearly related to the granular myelocytes and should probably be considered as the forerunners to these cells.

THE INFLUENCE OF THE REDUCTION OF KIDNEY SUBSTANCE UPON NITROGENOUS METABOLISM.[1]

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This communication presents the results of one of several investigations having for their object the determination of the part played by chemical correlation, in a broad sense, in the productions of pathological conditions associated with diseases of the kidney.[2] In taking up such problems one of the first questions for consideration is that of the influence of the hypothetical internal secretion of the kidney upon general nitrogenous metabolism. I use the term hypothetical advisedly, for although this theory occupies a prominent place in the literature dealing with the question of uraemia and is very suggestive, there is little experimental or other evidence to support it.

It was first brought to general notice by Brown-Sequard,[3] who as the result of his investigation of the internal secretion of the testicle came to very broad conclusions concerning internal secretions in general and stated that the kidney, as also other organs, have this function. He based his opinion as regards the kidney on clinical observation[4] and the study of dogs from which both kidneys had been removed as compared with those in which the ureters had been ligated. As the result of both procedures the retention of metabolites is the same, but in the nephrectomized animals, death, which comes on more rapidly, is supposed to be

[1] Aided by a grant from the Rockefeller Institute for Medical Research. Received for publication May 26, 1908.

[2] Pearce, R. M., The Theory of Chemical Correlation as Applied to the Pathology of the Kidney, Annual Address before the Philadelphia Pathological Society, April 23, 1908.

[3] Brown-Sequard, Importance de la secretion interne des reins, *Arch. d. phys. norm. et path.*, 1893, v, 778.

[4] Fowler, E. P., Suppression of Urine, New York, 1881.

due to the absence of an internal secretion which, it is assumed, is still furnished by the ligated kidneys. If, as he claimed, in the nephrectomized animals the internal secretion is replaced by the injection of renal juice, or glycerine extracts of kidney or by normal serum the animals live as long or even longer than those which have had both ureters ligated. Such observations are urged in support of the theory of internal secretion by those who do not believe uraemia to be due to the retention of the products of metabolism.

Similarly, metabolism experiments on man which tend to show that uraemia may occur without evident nitrogen retention are viewed in the same way and are taken by those who support the theory of internal secretion to indicate that uraemia is not due to lack of elimination of metabolites but to loss of the internal secretion.

From the experimental side the only method of attack has been by the study of the variation in elimination of nitrogenous substances after extirpation of amounts of kidney substance sufficient presumably to disturb the hypothetical internal secretion. Upon this phase of the subject the observations of Tuffier, of Bradford and of Bainbridge and Beddard are available. Tuffier[5] removed first one kidney, and after an interval, a portion of the other. He came to the conclusion, based on ten experiments, that 1.5 gram of kidney substance per kilo of body weight was sufficient to maintain life and adds that except for oscillations due to the immediate effects of the operation no changes in the elimination of urine or urea were noted.

Bradford[6] also working with dogs, found that after the removal of approximately three fourths of the total kidney weight death occurred in from one to six weeks from asthenia with great wasting; coma and convulsions were not observed. Death is apparently dependent on the amount of kidney substance removed and not upon mutilation inflicted by the surgical operation. Excision of a portion of one kidney or portions of both is followed by an increase in the

[5] Tuffier, T., Études expérimentelles sur la chirurgie des reins, Paris, 1889 (quoted from Bradford).

[6] Bradford, J. R., The Results following Partial Nephrectomy and the Influence of the Kidney upon Metabolism, *Jour. of Physiol.*, 1899, xxiii, 415.

TABLE I.

(*Dog 4, ¼ and ½ reduction.*)

Date.	Urine.							Faeces.		Weight. Gms.	Notes.
	Amt. c.c.	Sp. Gr.	Total N. Gms.	Urea. Gms.	Urea. Per Cent.	Ammonia. Gms.	Ammonia. Per Cent.	Total N. Gms.	Per Cent. N.		
Control Period.											
Oct. 21	525	1013	6.84	5.67	82.9	0.323	4.7	0.1285	3.0	13,780	
22	430	1017	6.45	5.35	83.0	0.320	4.9	0.1285	3.0	13,860	
23	545	1012	6.65	5.46	82.1	0.318	4.8	0.1285	3.0	13,840	
24	½ right kidney removed—wt. 25 gms.										
26	430	1016	8.25	7.09	86.0	0.338	4.1	0.1894	4.2	13,410	Albuminuria
27	430	1018	7.22	6.04	83.4	0.315	4.4	0.1894	4.2	13,400	
28	480	1014	7.81	6.53	83.5	0.293	3.8	0.1894	4.2	13,240	
Nov. 4	325	1020	6.69	5.62	85.5	0.231	3.5	0.2641	4.4	13,500	
5	440	1017	6.56	5.60	86.5	0.286	4.3	0.2641	4.4	13,770	Diarrhoea
6	430	1016	6.26	5.18	82.9	0.266	4.2	0.2641	4.4	13,610	
7	325	1018	5.25	4.34	82.6	0.240	4.6	0.2641	4.4	13,760	
8	460	1015	5.9	4.87	82.5	0.321	5.4	0.2641	4.4	13,840	Diarrhoea
8	½ left kidney removed—wt. 22 gms.										
20	410	1012	5.89	4.93	83.6	0.264	4.5	0.1765	3.07	11,810	
21	440	1013	5.65	4.83	85.6	0.291	5.1	0.1765	3.07	11,960	
22	510	1010	5.52	lost.		lost.		0.1765	3.07	11,930	100 c.c. extra water
Dec. 3	725	1008	7.14	6.14	85.9	0.343	4.8	0.6335 (?)	4.68	12,250	300 c.c. extra water
4	615	1009	6.39	5.31	83.1	0.298	4.7	0.6335 (?)	4.68	12,285	"
4	3d operation—death in 3 hours. Oedema of lungs.										

Weight of kidney removed at first and second operations.... 47 gms.
Weight of kidney found at autopsy..... 43.7 gms.

case of the former the remaining half of this kidney was taken out at a third operation. In some instances one entire kidney and half the opposite kidney were removed at one operation without any immediate ill effect. The present work includes metabolism studies

on four dogs with varying degrees of kidney reduction, but a somewhat exhaustive study of the general effects of extirpation and of

TABLE II.

(*Dog 21, ⅔ reduction.*)

Date.	Urine.							Faeces.		Weight Grams.
	Amount c.c.	Sp. Gr.	Total N. Grams.	Urea. Gms.	Urea. Per Cent.	Ammonia Gms.	Ammonia Per Cent.	Total N. Grams.	Per Cent. N.	
Dec. 3		Rt. kidney (wt. 29 gms.) ⅓ Lt. kidney (wt. 10 gms.)				Removed				8,410
17	440	1014	6.47	5.59	86.4	0.282	4.4	0.1806	4.34	8,210
18	437	1014	6.18	5.70	83.7	0.269	4.4	0.1806	4.34	8,230
19	435	1015	5.24	4.28	81.7	0.259	4.9	0.1806	4.34	8,225
Jan. 6		Killed								8,050

Weight of kidney removed at single operation..... 39 gms.
Weight of kidney remaining at autopsy..... 16.5 gms.

Total weight ..... 55.5 gms.

TABLE III.

(*Dog 12, spontaneous nephritis, unilateral nephrectomy.*)

Date.	Urine.							Faeces.		Weight Gms.	Notes.
	Amt. c.c.	Sp. Gr.	Total N. Gms.	Urea. Gms.	Urea. Per Cent.	Ammonia. Gms.	Ammonia. Per Cent.	Total N. Gms.	Per Cent. N.		
Nov. 16	250	1031	6.55	5.04	77.2	0.276	4.2	0.1514	2.93	17,820	Alb. 4 per cent. Casts +++
18	300	1028	6.71	5.39	80.4	0.290	4.3	0.1514	2.93	17,610	
19	212	1036	6.42	4.99	77.8	0.345	5.3	0.1514	2.93	17,520	
20	260	1034	6.66	5.22	78.4	0.362	5.4	0.1514	2.93	17,500	
22											Alb. 2¼ per cent. Casts +++
Dec. 6	Right kidney removed, weight 72 gms.							0.2058	3.58		
10								0.1981	3.44		Alb. 1½ per cent. Casts ++
11	405	1017	7.80	6.05	77.6	0.335	4.29	0.1981	3.44	17,110	300 c.c. extra water
12	505	1015	6.44	5.23	81.1	0.313	4.86	0.2108	3.59	17,100	300 c.c. extra water
16								0.2108	3.59		
20	Died during operation on other kidney, which weighed 68 gms.									16,650	

the process of repair in the kidney after various kinds of operative injury will be published later by Dr. J. A. Sampson and myself. At present it is sufficient to state that we have had no difficulty in keeping animals alive and in good condition without either general or local disturbances after the removal of one quarter, one half, and in some instances three quarters of the total kidney substance at one operation. The removal of larger amounts and occasionally of three quarters is followed by severe general disturbances which have rendered futile all attempts to maintain the animal in nitrogenous equilibrium. In one instance after removal of a considerable portion of the kidney substance, a nephritis developed, which added to the value of the experiment rather than otherwise.

TABLE IV.

(*Dog 13, 3/4 and 6/7 reduction; acute nephritis.*)

Date.	Urine.							Faeces.		Weight Gms.
	Amount c.c.	Sp. Gr.	Total N. Gms.	Urea. Gms.	Urea. Per Cent.	Ammonia. Gms.	Ammonia. Per Cent.	Total N. Gms.	Per Cent. N.	
Nov. 2	Half left kidney removed, weight 14.5 gms.									15,840
13	Entire right kidney removed, weight 42 gms.									14,000
16	Nephritis. Alb. = ½ per cent. Abundant casts.							0.2125	5.53	13,100
				5.29		0.166				
21	410	1011	5.72		92.5(?)		2.9			12,970
				4.84		0.251				
22	320	1016	4.78		80.2		5.2			13,070
				4.62		0.272				
23	375	1019	5.40		85.3		5.2			13,270
Dec. 3–8	Does not eat—more or less vomiting.									
				2.87		0.143				
6	267	1016	3.61		79.5		4.0	0.0990	5.74	12,680
				2.43		0.119				
7	222	1020	3.04		79.9		3.9	0.0990	5.74	12,590
9	Eight gms. of remainder of left kidney removed.									
10–20	Gastro-intestinal disturbance—does not eat. Alb. 1½–2 per cent. Casts abundant.									
16								0.1766	6.66	
				1.56		0.040				
18	165	1016	1.89		82.5		2.1	0.0557	6.65	10,350
				1.24		0.028				
19	120	1017	1.47		84.4		1.2	0.0557	6.65	10,200
				0.437		0.527				
20	195	1008	0.689		63.4		7.6	0.0557	6.65	10,000
21	Chloroformed.									

Weight of kidney substance removed..... 64.5 gms.
Weight of remaining kidney..... 11 gms.

Total weight ..... 75.5 gms.

The results are shown in Tables I, II, III and IV. As a normal control period is given in one experiment only (Table I) a series of controls[8] are presented in Table V. These give the figures in six dogs fed on the same diet and kept under the same conditions as the dogs with kidney reduction. The averages only are given and are based on the figures of periods of three to five successive days.

TABLE V.

Normal Dogs—Control.

Dog.	Total N. Gms.	Urea.		Ammonia.		Creatinin.		Undetermined N.		Average of
		Gms.	Per Cent.	Gms.	Per Cent.	Gms.	Per Cent.	Gms.	Per Cent.	
A	6.35	4.96	78.1	0.019	7.9	0.606	9.5	0.259	4.1	Five 24° periods.
B	3.30	3.24	86.1	0.128	3.3	0.278	7.4	0.108	2.9	Four " "
C	4.10	3.98	78.4	0.450	8.8	0.350	6.7	0.326	6.0	Four " "
D	7.04	5.39	81.2	0.395	5.5	0.345	4.8	0.568	8.1	Three " "
E	6.56	5.30	80.7	0.428	5.8	0.281	4.2	0.537	7.9	Three " "
F	6.57	5.61	85.3	0.364	5.5	0.354	5.4	0.213	3.4	Three " "
G	5.36	4.34	82.0	0.332	6.7	0.208	3.9	0.377	7.4	Four " "
Average Per Cent.			81.7		6.2		6.0		5.7	

Experiment I shows no appreciable changes in metabolism after the removal of one half of one kidney or one half of each kidney. The animal unfortunately died a few hours after the third operation with no lesions discoverable at autopsy except a very extensive oedema of the lungs.

Experiment II indicates that the removal of two thirds of the entire kidney substance at one operation does not interfere with the general condition of the animal as shown by the constant weight and normal metabolism figures.

The third experiment made on an animal with a spontaneous nephritis, a condition occasionally found in stray dogs, shows that

[8] Taken from paper by Pearce, R. M., and Jackson, H. C., Experimental Liver Necrosis; III, Nitrogenous Metabolism, *Jour. Exper. Med.*, 1907, ix, 552.

this lesion has no effect on metabolism, even after the kidney substance is reduced one half by a unilateral nephrectomy.

The fourth experiment was not planned for a study of metabolism, but when it was found that an animal with but one quarter of its kidney substance had developed an acute nephritis, thus diminishing, it was assumed, the functional capacity of the fractional portion of the kidney remaining, it seemed too good an opportunity to lose and the animal was placed on a constant diet and the metabolism experiments carried out six days later. This animal, despite the great reduction of kidney substance and the presence of a nephritis, had a normal metabolism during the first three day observation period. Two weeks later, however, when appetite had begun to fail equilibrium was lost, although no change in the percentage relations of nitrogen was evident.

But after another period of two weeks had elapsed, and the kidney substance had still further been reduced by operation, leaving the animal with but one seventh of its original kidney material, a change in the urea-ammonia ratio, indicative of inanition, occurred. That this final change is due, as Bainbridge and Beddard claim, to inanition there can be no doubt. Up to this point, the beginning of starvation, the changes in urea described by Bradford were not observed.

The discrepancy between these results and those of Bradford require a word of comment. Bradford, it will be remembered, found an increase of urea to occur with considerable frequency after removal of one half or two thirds of the kidney substance and almost constantly after removal of three fourths. In the experiments here quoted the urea remained constant until the period of inanition. In my experiments exact twenty-four-hour samples of urine were obtained by catheterization, the animals were kept on a constant diet (casein, lard, cracker dust) and the more exact Mörner-Sjoquist method for the determination of urea was used. Bradford's estimations on the other hand were based on the amount of urine voided naturally in twenty-four hours; the diet (meat and biscuit) was weighed accurately but was administered according to the appetite of the animal; and the urea was estimated by the relatively inaccurate hypobromite method. The differences in methods explain, I think, the variance in results.

Creatinin estimations were not made in these experiments on account of the negative results obtained by Bainbridge and Beddard. The undetermined nitrogen was therefore not calculated, but if the averages for creatinin in normal dogs, presented in Table V, are taken as indicating the probable creatinin excretion and added to the urea and ammonia percentage it is found that the undetermined nitrogen does not in any instance, except in the dog with spontaneous nephritis (Table III) and in the later period of Dog 13 (Table IV), vary widely from the normal average of 5.7 per cent. In this regard the nitrogen determination would therefore also appear to be normal. As far as the urine is concerned it would appear that no evidence is at hand to indicate that the kidney, through an internal secretion or otherwise, has any influence on general nitrogenous metabolism and I believe the theory of internal secretion in this regard at least may be set aside. There remains, however, the very important question of why the removal of more than three fourths of the kidney substance leads to loss of appetite and consequent inanition. Although vomiting occasionally occurred in these animals it did not seem to be sufficiently frequent or severe to indicate a general gastro-intestinal disturbance. To test this point extirpation of the kidney was done on two dogs after the establishment of a gastric fistula. In this way it was hoped that the food necessary for nitrogenous equilibrium could be introduced artificially and by examination of both urine and fæces some light might be thrown on the cause of the disturbance. These efforts were rendered futile, however, by the inability of the stomach to retain the materials introduced. The conclusion is inevitable therefore that although the kidney appears to have no direct influence on nitrogenous metabolism the removal of large portions of its substance does indirectly lead to disturbances of general nutrition by interfering apparently with the functions of the alimentary canal.

Examination of the Fæces.—The occurrence of serious gastric and intestinal disturbances in these animals after kidney reduction and their general similarity to lesions occurring occasionally in man in the course of chronic nephritis led me, in view of our more recent ideas of the chemical control of the body and the influence of

one secretion on another, so well brought out by Starling and Baylis's investigations, to study the fæces of the animals in order to determine if partial nephrectomy had any influence on digestion, absorption or elimination into the intestine. Brainbridge and Beddard's claim that the disturbance in metabolism is due to inanition dependent on vomiting and diarrhœa with eventual failure to take food and not to a direct influence of the kidney dodges the question. The point to be determined is whether or not the gastric and intestinal disturbances are caused by faulty absorption or by digestive disturbances due to the elimination into the intestine of products normally removed by the kidneys. The demonstration of such a relation would be important not only as an illustration of the chemical interrelationship between various organs, but would also aid in explaining similar disturbances associated with the nephritis of man.

These latter which, I understand, are frequently so severe as to present symptoms closely resembling a violent gastro-enteritis have been ascribed in part to alterations of the mucosa due to oedema, and in part to the influence of the uræmic condition on the central nervous system. While these explanations account for many of the symptoms, others, according to von Noorden,[9] must be attributed to toxic chemical action. In fact recent investigations show that in uræmia substances usually eliminated by the kidneys are secreted vicariously into the alimentary tract. "Of these, the most irritating chemically is ammonia, which is formed in the intestine by decomposition of the secreted urea. It is also noticeable that the fæces in uræmic diarrhœa are extremely rich in ammonia."

Studies of the fæces having for their object the determination of the degree of absorption in nephritis, for which we are indebted mainly to von Noorden, show that the absorption of fats is very complete. The situation in regard to nitrogen is not so clear, the loss in some cases being greater than normal while in others an abnormally high percentage is found. The variation in some individuals, without a corresponding change in diet, or in the nature of the stools, or in the general condition and with no

[9] Von Noorden, C., Metabolism and Practical Medicine (Vol. ii). Chicago, 1907.

change in the percentage of dry substance or fat content of the fæces, led von Noorden to the conclusion that the increase of nitrogen was due not to impaired absorption, but to the vicarious excretion of metabolites stored up in the organism. In many cases the excretion remained normal. High amounts, above three grams daily, were found in nephritis only in diarrhœa (uræmic) and were due largely to a high content of ammonium salts, constituting sometimes ten to twenty per cent. of the total fæcal nitrogen.

In the four experiments tabulated above the total nitrogen in the fæces evacuated during control periods and periods of metabolism study was estimated. The results as seen in the tables indicate no marked change in the total nitrogen or its percentage relation, except possibly in Experiment IV. In this animal with but one seventh of its kidney substance, which after the first operation developed a severe nephritis, the *percentage* of nitrogen in the fæces apparently shows a somewhat marked increase as compared with the other dogs. Unfortunately, no control figures on this animal are at hand. The low total nitrogen combined with a high percentage would appear to indicate the presence of substances of a higher nitrogen percentage content (*e. g.*, ammonia) than those normally present in the fæces. This phase of the problem will be further investigated.

The inanition due to gastro-intestinal disturbance cannot therefore be explained by impaired absorption, or, except as possibly indicated by Experiment IV, by an undue elimination of nitrogenous substances. Irritation by increased elimination of toxic substances, non-nitrogenous in nature, may be a factor, but upon this point I have as yet no observations.

CONCLUSIONS.

1. The removal of one half, two thirds and sometimes three quarters of the kidney substance in the dog causes no change in the general nitrogenous metabolism as determined by estimations of the total nitrogen, urea and ammonia elimination by the urine.

2. The removal of larger amounts, and sometimes of three quarters of the substance, leads to the metabolism condition of starvation. This, however, is apparently the result of the gastro-

intestinal disturbance constantly associated with extensive kidney reduction and not of a disturbance of general nitrogenous metabolism.

3. The determination of the amount of fæcal nitrogen indicates that the gastro-intestinal disturbance is not due to diminished absorption; and except in one instance there was no evidence of its being due to an increased elimination of nitrogenous substances into the intestine.

4. These experiments do not support the theory that the kidney furnishes an internal secretion having an important influence on general nitrogenous metabolism. At least, if such a function exists, it is not disturbed by the removal of three quarters of the kidney substance.

5. The metabolism in excessive kidney reduction is that of inanition dependent on gastro-intestinal disturbances presumably due to faulty chemical correlation. In this connection further knowledge concerning the elimination into the intestine of toxic substances is desirable.

ENZYMES OF TUBERCULOUS TISSUE.*

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Although a large part of the tubercle consists of cells which act as phagocytes receiving into their cell body tubercle bacilli, little effort has been made to determine what enzymes are present in tuberculous tissue. There is little direct evidence of the occurrence of enzymes capable of digesting proteid, but study of the chemistry of tuberculous tissue has indirectly suggested the possibility that autolysis may occur within the tubercle.

Friedrich Müller[1] has found that both pulmonary tissue with tuberculous pneumonia and caseous material, unlike the gray hepatized lung of acute croupous pneumonia, undergo very little autolysis. Matthes[2] has obtained reactions indicating the presence of deutero-albumose and peptone in tuberculous glands which have not undergone complete caseation. Speithoff,[3] continuing the studies of Matthes, has found albumose and peptone, even though in very small amount, in all of ten extracts prepared from the non-caseous, mixed with the caseous, parts of tuberculous lymphatic glands of cattle, and has suggested that their presence may be referable to autolysis. In seven of ten instances in which caseous material has been carefully freed from non-caseous tissue, these substances have not been found, whereas in the remaining three specimens incomplete evidence of their occurrence have been obtained. Schmoll[4] has found that caseous material consists of protein with an elementary composition resembling that of albumin or globulin, but since it is almost wholly insoluble it is, he thinks, a coagulated proteid. Since phosphorus has been found to be scant

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[1] Müller, Fr., *Verhandl. d. 20 Cong. f. inner. Med.*, 1902, 192.

[2] Matthes, *ibid.*, discussion of address of Fr. Müller.

[3] Speithoff, *Zent. f. inner. Med.*, 1904, xxv, 481.

[4] Schmoll, *Deutsches Arch. f. klin. Med.*, 1904, lxxxi, 163.

in the caseous substance it has seemed probable that coagulative necrosis which is associated with destruction of nuclei allows the escape of products which are formed, for the change occurs in a tissue which is rich in phosphorus.

Heile[5] has suggested that failure of caseous tuberculous lesions to undergo absorption is due to absence of enzymes rather than to insolubility of caseous material. He has found that pus-like material from a cold abscess fails to digest fibrin, but after injection of iodoform in glycerine polynuclear leucocytes appear and the exudate dissolves fibrin. Edw. Müller and Jochmann[6] have found that pus-like fluid from a cold abscess, unlike pus of acute inflammation, is incapable of dissolving Löffler's blood serum when incubated at from 55 to 60° C., and Kolaczek and Edw. Müller[7] have claimed that failure of a drop of pus-like fluid to cause a depression upon the surface of a plate of Löffler's serum may be used for diagnosis of uncomplicated tuberculous lesions.

Those exudates and tissues which contain in abundance polynuclear leucocytes with fine granulation contain an enzyme which is distinguishable by its ability to digest proteid in an alkaline and in a neutral medium; these cells, it is well known, constitute the predominant element of most inflammation exudates. One of us has shown that the large mononuclear phagocyte, which is abundant in the later stages of many inflammatory reactions, contains an active enzyme which is distinguishable from that of the polynuclear leucocyte by its ability to digest proteid in the presence of a weakly acid reaction.[8] The purpose of the present study has been to determine if similar enzymes exist in tuberculous tissue.

Polynuclear leucocytes occur in scant number in the uncomplicated tuberculous lesions usually observed at autopsy and it has been believed by many observers that they have little part in the tuberculous process. Numerous experimental studies have shown that tubercle bacilli, like other bacteria, when injected into animals, cause an accumulation of polynuclear leucocytes which ingest and surround the microörganism. Benda[9] has shown that the same pro-

[5] Heile, *Zeit. f. klin. Med.*, 1904, lv, 508.

[6] Müller and Jochmann, *Münchener med. Woch.*, 1906, liii, 1393.

[7] Kolaczek and Müller, *ibid.*, 1907, liv, 253.

[8] Opie, *Jour. of Exper. Med.*, 1905, vii, 316.

cess occurs with human tuberculosis; he has studied the formation of miliary tubercles which occur with acute miliary tuberculosis when plugs of tubercle bacilli lodge in the capillary vessels of the renal glomeruli and has observed as the first result of the presence of the microörganism accumulation of polynuclear leucocytes, about the microörganism.

Polynuclear leucocytes quickly disappear from experimental or from human tuberculous tissue and give place to mononuclear cells, the so-called epithelioid cells which are the chief constituent of the fully developed tubercle. The well-known controversy concerning the origin of these cells is as yet unsettled. Baumgarten on the one hand has maintained that they are derived from the fixed cells of the tissue, whereas Metchinkoff, who has given especial attention to their behavior as phagocytes, has believed that they are derived from leucocytes and are identical with the large monouclear phagocyte or macrophage, which has a part in other forms of inflammation. Each view has been subjected by those who have supported it to a wide range of modification.

In order to determine if the proteolytic enzymes which occur in the polynuclear leucocytes and large mononuclear phagocytes of inflammatory exudates are present as well in tuberculous lesions it has been necessary to obtain tuberculous tissue in considerable quantity free from microörganism other than tubercle bacilli. The possibility of secondary infection of tuberculous material obtained at autopsy can be excluded by attempted cultivation of bacteria only after tissue has been prepared for the enzymic tests.

Masses of tuberculous tissue are formed in the mediastinum of the dog after injection of tubercle bacilli into one pleural cavity. At the end of two weeks the entire mediastinum and adjacent subpericardial membranes may be occupied by masses of pearly white translucent tissue which is firm but very succulent. At a later stage caseation occurs in this tissue which in large areas loses its translucent appearance, becomes opaque and yellowish white and is much less juicy than before the change has occurred. At a late stage of the disease caseation of the tuberculous tissue may be almost complete. The histology of the newly formed tuberculous tissue ob-

[9] Benda, Path.-anat. Arb. J. Orth gewidmet, p. 520.

tained at various intervals after inoculation and employed in the following experiments will be described in order to determine what relation exists between the enzymic activity of the tissue and the presence of certain types of cell.

Former studies[10] have shown that autolysis of tissues and cells when used in the small quantities in which most pathological products can be obtained gives an incomplete conception of the proteolytic enzymes which they contain. More accurate knowledge may be gotten by allowing a small quantity of the cells to be tested to act upon a relatively large quantity of denaturalized proteid; this method makes it possible to vary within wide limits the character of the medium in which digestion occurs. By the Kjeldahl method for the determination of nitrogen the amount of nitrogen of coagulable proteid converted into incoagulable substances is estimated. Masses of tuberculous tissue freed from fat have been finely divided by passing twice through a miniature sausage machine, which compresses the tissue and in large part squeezes out its juice. The mass of finely divided particles has been further freed from tissue juice by pressure between small pads of sterile gauze. The power of this material to cause proteolysis of heated blood serum has been tested in various strengths of acetic acid and of sodium carbonate. The method employed has been fully described in previous articles.

In the following experiments (Experiments 1 to 6) 0.3 grm. of tuberculous tissue freed from tissue-juice has been allowed to act upon denaturalized blood serum (ten cubic centimeters of a mixture of equal parts of blood serum and 0.85 per cent. sodium chloride heated one half hour at 75° C.) in the presence of acid, approximately neutral and alkaline media. The degree of digestion is indicated by cubic centimeters of one-tenth normal sulphuric acid.

EXPERIMENT 1. Eighteen days after inoculation of the right pleural cavity with an emulsion of tubercle bacilli the mediastinum contained masses of firm pearly white tissue. *Microscopical Examination.*—The tissue which is well vascularized is composed in large part of large mononuclear cells usually collected into small groups containing no blood vessels and surrounded by loose fibrous stroma. These cells have a large vesicular nucleus and abundant protoplasm. Mingled with them in small number are lymphoid and plasma cells. Polynuclear

[10] Opie, *Jour. of Exper. Med.*, 1905, vii, 759; 1906, viii, 410.

leucocytes which are numerous are scattered throughout the tissue and occur in small groups. The only suggestion of caseation is the occurrence of necrosis in a few foci of large mononuclear cells; here some of the cells have lost their nuclei and the cell body is broken in fragments. In such foci polynuclear leucocytes are especially numerous.

Tuberculous Tissue from Mediastinum.

	Control.	Acetic acid.		Neutral.	Sodium carbonate.	
		0.5 per cent.	0.2 per cent.		0.2 per cent.	0.5 per cent.
0.3 grm. tuberc. tissue + coagulated proteid	3.35	14.9	17.7	16.25	21.35	13.0

Studies of exudates and of various tissues of the normal animal, the pancreas excluded, have shown that active digestion in the presence of an alkaline medium is peculiar to inflammatory exudates containing polynuclear leucocytes and to tissues such as the bone marrow which contain these cells in large number. Since this enzyme is inhibited by a weakly acid medium occurrence of active digestion in the presence of acid indicates that a second enzyme is present. Evidence of the independent occurrence of this enzyme in tuberculous tissue will be furnished by subsequent experiments.

The following experiment confirms that just described and shows that tuberculous tissue obtained soon after onset of the disease can cause autolysis in the presence of acid and of alkali:

EXPERIMENT 2. An animal killed eighteen days after intrapleural injection of tubercle bacilli has exhibited advanced tuberculosis of the mediastinum and of the adjacent substernal lymphatic glands. *Microscopical Examination of Mediastinal Tissue.*—The predominant elements in the tissue are large mononuclear cells; lymphoid and plasma cells are fairly numerous. Throughout the tissue are numerous polynuclear leucocytes. The tissue is well vascularized and there is newly formed fibrous stroma. There is no caseation. *Hypertrophied Tuberculous Lymphatic Gland.*—The lymphoid tissue of the gland is in great part replaced by large mononuclear cells with vesicular nucleus and abundant protoplasm resembling those of the mediastinal tissue. Polynuclear leucocytes are very numerous throughout the gland. Fibrous stroma is increased. There is no caseation.

Tuberculous Tissue of Mediastinum and Hypertrophied Tuberculous Lymphatic Glands.

	Control.	Acetic acid. 0.2 per cent.	Neutral.	Sod. carb. 0.2 per cent.
0.3 grm. tuberc. tis. of mediastinum + coagulated proteid	4.0	15.45	12.75	14.65
0.3 grm. tuberc. lymph. gland + coagulated proteid	3.35	15.65	12.15	10.45

Digestion in acid and in neutral media is almost identical for the mediastinal and for the glandular tissue, but in an alkaline medium there is greater variation.

For the following experiment tuberculous tissue was obtained from an animal in which the disease was more advanced (twenty-nine days) than in the two foregoing experiments:

EXPERIMENT 3. Tuberculous tissue from the mediastinum has been obtained twenty-nine days after intrapleural inoculation with *B. tuberculosis*. This tissue is firm and in part resembles dense fibrous tissue but over more than one half of the cut surface is opaque, white, and apparently caseous. *Microscopical Examination.*—The tissue is in considerable part necrotic and nuclear stain is absent but throughout occur scattered areas of well preserved tissue which almost always surround an artery of considerable size. In these areas mononuclear cells make up the greater part of the tissue; these cells are usually somewhat smaller more irregular in shape and less clearly outlined, the one from another, than the cells seen at an earlier stage of the lesion. They resemble closely the epithelioid cells of human tubercle. Fibrous stroma is abundant. Polynuclear leucocytes are scant but a few small collections of such cells occur.

Tuberculous Tissue of Mediastinum.

	Control.	Acetic acid.			Neutral.	Sodium carbonate.		
		1 per cent.	0.5 per cent.	0.2 per cent.		0.2 per cent.	0.5 per cent.	1 per cent.
0.3 grm. tuberc. tis. + coag. proteid..	3.05	10.35	11.75	12.25	12.6	4.35	5.15	6.25

Digestion in the presence of acid has been active, whereas in the presence of weak alkali (0.2 per cent.) digestion has been almost wholly absent; the somewhat larger figures obtained with higher strength of alkali (0.5 and 1 per cent.) are probably referable to increased difficulty in causing complete coagulation of undigested proteid by heat in the presence of stronger alkali even after neutralization. Absence of digestion in the presence of 0.2 per cent. sodium carbonate indicates absence of leucoprotease and is in accord with the almost complete absence of polynuclear leucocytes in the tissue which has been used.

Almost identical results have been obtained with tuberculous tissue from the omentum obtained sixty-nine days after intraperitoneal inoculation with a strain of tubercle bacillus more virulent for rabbits and for dogs than that previously employed.

EXPERIMENT 4. Sixty-nine days after intraperitoneal inoculation with *B. tuberculosis* the omentum is represented by a firm flat mass of tissue about 1 cm. in thickness; this tissue is in part grayish-white but in greater part opaque and caseous in appearance. *Microscopical Examination.*—Fat of the omentum is replaced by tissue which is formed by large mononuclear cells together with a few lymphoid and plasma cells. Polynuclear leucocytes are almost wholly lacking except at the margin of caseous tissue where a few are found. Caseous areas of considerable size occur; the large mononuclear cells lose their nuclei, their protoplasm stains deeply and homogeneously and finally breaks into particles. Coarse strands of newly formed fibrous tissue are present.

Tuberculous Tissue from the Omentum.

	Control.	Acetic acid.			Neutral.	Sodium carbonate.		
		1 per cent.	0.5 per cent.	0.2 per cent.		0.2 per cent.	0.5 per cent.	1 per cent.
0.3 grm. tuberc. tis. + coag. proteid..	3.8	15.85	16.5	17.15	11.9	7.15	4.9	4.45

The following experiment in which tuberculous tissue obtained after long continued tuberculosis (forty-seven days) has been used furnishes evidence that proteolyic enzymes tend to disappear after the onset of caseation:

EXPERIMENT 5. Forty-seven days after intrapleural inoculation with *B. tuberculosis* the mediastinum contains masses of tissue which has undergone almost complete caseation. The substernal lymphatic glands are greatly enlarged and homogeneously caseous. In the mediastinum behind the substernal lymphatic glands are two enlarged lymphatic glands which are translucent grayish white and succulent showing little if any caseation. An hypertrophied gland situated near the duodenum has the same appearance and together with those just mentioned has been used in the tests which follow. *Microscopical Examination of Caseous Mediastinal Tissue.*—Caseation with complete absence of nuclear stain occurs throughout the tissue save immediately below its surface where a capsule of fibrous tissue contains a few large mononuclear and lymphoid cells. *Microscopical Examination of Caseous Substernal Lymphatic Gland.*—Caseation completely obscures all structure save in a few small foci about blood vessels where large mononuclear and lymphoid cells occur. Polynuclear leucocytes are almost entirely absent. *Microscopical Examination of Hypertrophied Tuberculous Lymphatic Gland.*—Cells which resemble the large mononuclear cells of tuberculous tissue elsewhere occur in the sinuses and almost wholly replace the cords of lymphoid tissue which are well defined in only a small part of the tissue. The peripheral sinus of the gland is occupied by large mononuclear cells which resemble on the one hand the large mononuclear cells of tuberculous tissue and on the other hand the large phagocytic cells of the lymphatic gland (the so-called endothelial cells). These cells are closely packed together and in places have undergone degenerative changes; the nucleus is pale and swollen or has completely disappeared; the cell body stains deeply, loses its well-defined outline and may break into fragments. Polynuclear leucocytes are present in considerable number throughout the gland.

Caseous tissue used in the following tests has been obtained from the mediastinum and substernal lymphatic glands; the hypertrophied tuberculous glands examined for comparison are those mentioned.

Caseous Material and Hypertrophied Tuberculous Glands.

	Control.	Acetic acid. 0.2 per cent.	Neutral.	Sod. carb. 0.2 per cent
0.3 grm. caseous tuberc. tis. + coag. proteid	2.65	7.6	9.00	2.6
0.3 grm. hypertrophied tuberc. gland + coag. proteid	2.85	14.9	10.15	10.95

With advance of tuberculosis and occurrence of widespread caseation leucoprotease indicated by digestion in an alkaline medium has almost completely disappeared. In the hypertrophied gland the lesion is much less advanced and digestion still occurs in an alkaline medium. Comparison of the caseous tissue with the hypertrophied lymphatic gland and with tissue used in previous experiments in which tuberculosis has been less advanced shows that the enzyme capable of digesting in an acid medium has dimininshed considerably. In the following experiment caseation is almost complete and enzymes, measured by ability of the tissue to cause proteolysis, have almost wholly disappeared.

EXPERIMENT 6. After inoculation with *B. tuberculosis* fluid had accumulated in the right pleural cavity. Slightly turbid, almost colorless fluid had been withdrawn for use in experiments to be described later. The animal was killed with ether sixty-five days after inoculation.

The right pleural cavity contains 175 c.c. bloody fluid; the left cavity contains 134 c.c. reddish serous fluid. The mediastinum is occupied by bulky masses of hard, opaque, yellow tissue. *Microscopical Examination of Mediastinal Tissue.*—Tissue is wholly caseous save for several minute patches in which mononuclear cells with fairly abundant protoplasm occur in fibrous stroma; a few polynuclear leucocytes are present.

Caseous Mediastinal Tissue.

	Control.	Acetic acid.		Neutral.	Sodium carbonate.	
		0.5 per cent.	0.2 per cent.		0.2 per cent.	0.5 per cent.
0.3 grm. caseous tuberc. tis. + coag. proteid...	2.25	6.8	6.6	6.6	2.85	3.45

Tuberculous tissue of the dog during the early stages of its formation contains polynuclear leucocytes in considerable number and exhibits ability to cause proteolysis under conditions which characterize the enzyme of these cells. At a later period polynuclear leu-

cocytes disappear and power to digest in the presence of alkali is lost.

The enzyme which has been constantly found in tuberculous tissue and is present alone during the later stages of the disease is identified by its ability to cause proteolysis in an acid and in a neutral medium, whereas it is wholly inhibited by weak alkali. An enzyme of the same character, designated for convenience lympho-protease, is present in the large mononuclear phagocytes which occur in inflammatory exudates. In the dog the large mononuclear cell, the so-called epithelioid cell of the tubercle, is identical in structure with the mononuclear phagocyte or macrophage of the inflammatory exudate. This mononuclear cell of the tubercle is actively phagocytic, ingesting tubercle bacilli and occasionally taking into its substance polynuclear leucocytes and other cells.

Experiments 1 and 2 illustrate the coincident action of the two enzymes which have been mentioned. Experiments with leucoprotease described in previous articles show that this enzyme causes almost equal digestion in a neutral medium and in 0.2 per cent. sodium carbonate, but little if any in acid. Experiment 3 shows that the enzyme of tuberculous tissue when almost unaccompanied by leucoprotease (indicated by digestion in alkali) may cause nearly equal activity in neutral and in acid (0.2 per cent. acetic acid) media. Nevertheless when the two enzymes act together in the presence of a neutral medium (Experiments 1 and 2) digestion by the two in combination is not greater, but is less than that by the same tissue in alkali or in acid. This fact suggests that when the two enzymes are brought together under conditions which permit the activity of both, one partially inhibits or perhaps partially destroys the other.

Comparison of the different experiments which have been described is possible because digestion caused by tuberculous tissue has been tested under almost identical conditions. Since leucoprotease is almost wholly inactive in the presence of acid, digestion in 0.2 per cent. acetic acid may be used an an index of the content of enzyme capable of digesting in acid. In the table which follows are collected results previously obtained when 0.3 grm. of tuberculous tissue from the same situation, namely, the mediastinum, has been

allowed to act upon a constant quantity of coagulated blood serum in the presence of 0.2 per cent. acetic acid; the figure representing the control has been subtracted from that obtained after incubation during five days at 37° C. and the difference measures digestion of proteid caused by tuberculous tissue obtained at various intervals after inoculation.

Results of Experiments with Mediastinal Tissue in Acid.

Exper. 1. 18 days.	Exper. 2. 18 days.	Exper. 3. 29 days.	Exper. 5. 47 days.	Exper. 6. 65 days.
14.35	11.45	9.2	4.95	4.35

When during the third week after inoculation the figure indicates a maximum degree of digestion caseation is beginning. At this time it affects almost exclusively the large mononuclear cells which lose their nuceli, become hyaline and break into particles. The possibility is suggested that caseation may be referable to autolysis. Subsequent experiments will show that the normal power of the blood serum to check the enzymic action of tuberculous tissue may be considerably diminished in a tuberculous exudate.

After onset of caseation there is gradual and probably complete disappearance of enzymes. It is probable that the small amount of digestion recorded in Experiments 5 and 6 is referable to minute islands of living tissue rich in epithelioid still persisting in the midst of almost complete necrosis.

Various tissues of the body have been found to cause more active proteolysis in acid than in alkali. Biondi,[11] Hedin and Rowland[12] and other observers have shown that autolysis of tissue from the liver, spleen, kidney and other organs is more active in the presence of weak acid than in neutral or in alkaline media. Examination of the liver containing miliary tubercles in great number has afforded an opportunity of comparing under almost identical conditions the enzymic activity of tuberculous tissue with that of a parenchymatous organ. The liver tissue of four animals has been employed; one has been obtained from a normal animal and three are tuberculous, obtained eighteen and sixty-five days after inoculation.

[11] *Virchow's Arch.*, 1896, cxliv, 373.
[12] *Zeit. f. physiol. Chem.*, 1901, xxxii, 341.

EXPERIMENT 7.

Proteolysis Caused by Hepatic Tissue Containing Miliary Tubercles.

	Control.	Acetic acid. 0.2 per cent.	Neutral.	Sod. carb. 0.2 per cent.
Normal Liver	2.6	10.25	6.5	2.9
A Liver with small tubercles (18 days)	2.45	13.75	10.2	4.35
B Liver with large tubercles (18 days)	2.7	17.1	8.9	5.05
C Liver with large tubercles (65 days)	2.55	15.8	5.3	4.0

Microscopical Examination of Tuberculous Livers. A.—About half of the section is occupied by small miliary tubercles composed of large mononuclear cells loosely packed together; a considerable number of polynuclear leucocytes occur among the cells. There is no caseation. B.—Considerably more than half of the section is occupied by large miliary and conglomerate tubercles composed of loosely packed mononuclear cells together with polynuclear leucocytes in considerable number. Caseation is beginning and the centers of many tubercles are necrotic. C.—More than half of the section is occupied by large tubercles composed of closely packed mononuclear cells; a moderate number of polynuclear leucocytes are present. There is no caseation.

The presence of miliary tubercles has increased proteolytic activity in all media, but especially in acid. The increased digestion which has been observed is significant, for it is well known that the activity of digestion does not increase in direct proportion to the quantity of enzyme, but bears a closer relation to the square root of the quantity of enzyme. Furthermore it is probable that the method does not permit digestion above a certain maximum for the accumulation of products of digestion doubtless inhibits the process. Increase of digestion is greatest in the presence of acid; the following figures show the amount of digestion represented by the difference between the control and the incubated preparation.

Results of Experiments with Liver in Acid.

Normal Liver.	Liver A.	Liver B.	Liver C.
7.65	11.3	14.4	13.25

It has been shown by one of us[18] that the serum of the blood is capable of inhibiting the enzyme which is contained in the polynuclear leucocytes. By use of the Kjeldahl method for estimation of nitrogen the influence of anti-enzyme upon a constant quantity of enzyme can be measured with considerable accuracy. This anti-enzymotic property of the blood is transmitted to the serum of an

[18] Opie, *Jour. of Exper. Med.*, 1905, vii, 316.

inflammatory exudate and doubtless holds in check enzyme set free by disintegration of polynuclear leucocytes, thus preventing an injurious action upon tissue with which the enzyme comes in contact. A year later Stern and Eppenstein[14] demonstrated the same fact by showing that leucocytes from the blood of patients suffering with spleno-myelogenous leukæmia liquefy gelatin, but lose this power in the presence of blood serum. Edw. Müller and Jochmann[15] subsequently repeated the observation and showed that the power of polynuclear leucocytes from various sources to cause a depression upon the surface of coagulated blood serum is lost in the presence of fresh serum of the blood.

In an article published in 1906, one of us[16] has shown that the anti-enzymotic activity of serum from the blood and from a serous inflammatory exudate is absent in the serum of a purulent exudate. Experiments have furnished evidence that the enzyme of the polynuclear leucocytes is present in serum of the abscess in such excess that the anti-enzymic power of this serum is overcome. Absence of anti-enzyme is believed to explain the solution of fibrin and of tissue which is characteristic of suppuration. The observation just described has been confirmed by Müller and Kolaczek,[17] who have used coagulated serum plates.

In view of the relation of the serum of the blood and of inflammatory exudates to the enzyme of the polynuclear leucocytes, it is desirable to study the behavior of the enzymes of tuberculous tissue in the presence of the serum of a tuberculous exudate. After injection of tubercle bacilli into the pleural cavity of the dog fluid accumulates within the chest and formation of tuberculous tissue upon the pleural membranes and in the mediastinum is associated with gradual increase of fluid. Repeated attempts to cultivate bacteria from this fluid have been unsuccessful.

The following experiment shows that the serum of a tuberculous exudate inhibits in slight degree digestion caused by tuberculous tissue.

[14] Stern and Eppenstein, *Vortrag in der Schles. Gesellsch. f. Vaterland. Kultur,* June 29, 1906. Quoted by Eppenstein, *Münchener med. Woch.,* 1906, liii, 2192.

[15] Müller and Jochmann, *Münchener med. Woch.,* 1906, liii, 1507.

[16] Opie, *Jour. of Exper. Med.,* 1906, viii, 410.

[17] Müller and Kolaczek, *Münchener med. Woch.,* 1907, liv, 354.

EXPERIMENT 8. Tuberculous mediastinal tissue has been obtained fourteen days after inoculation. The exuded tuberculous serum has been obtained by centrifugalization of a tuberculous pleural exudate withdrawn from the chest cavity seventy-one days after inoculation with tubercle bacilli. As in previous tests 0.3 grm. of tissue freed from juice has been allowed to act upon the usual quantity of heated serum.

Effect of Serum of Tuberculous Pleural Exudate upon Digestion Caused by Tuberculous Tissue.

	Control.	After 5 days at 37° C.	Digestion.
Tuberc. tis. + coag. proteid	3.3	13.85	10.55
Tuberc. tis. + 0.2 c.c. exuded tuberc. ser. + coag. p.	3.35	11.95	8.6
Tuberc. tis. + 1 c.c. exuded tuberc. ser. + coag. p.	3.6	11.9	8.3

To obtain more information concerning the degree of inhibition exerted by serum of a tuberculous exudate upon enzymes contained in tuberculous tissue comparison has been made between the serum of a tuberculous pleural exudate and the serum of blood.

EXPERIMENT 9. A part of the tuberculous tissue used in the previous experiment has been ground and freed from juice; it has been suspended in physiological salt solution (1.4 grm. in 70 c.c.) and after addition of toluol (3 c.c.) incubated at 37° C. during two days. Ten cubic centimeters of this solution filtered through cheese cloth has been allowed to act upon coagulated proteid in the presence of serum of a tuberculous pleural exudate withdrawn seventy-three after inoculation and in the presence of serum of the blood.

Effect of Serum of Tuberculous Pleural Exudate and of Serum of Blood upon Digestion Caused by Extract of Tuberculous Tissue.

	Control.	After 5 days at 37° C.	Digestion.
Extract of tuberc. tis. + coag. proteid	4.8	16.95	12.15
Extract of tuberc. tis. + 2.5 c.c. exuded tuberc. ser. + c. p.	5.3	11.95	6.65
Extract of tuberc. tis. + 5 c.c. exuded tuberc. ser. + c. p.	5.	13.8	8.0
Extract of tuberc. tis. + 2.5 c.c. blood serum + c. p.	5.4	5.15	0
Extract of tuberc. tis. + 5 c.c. blood serum + c. p.	6.1	6.0	0

Two facts are noteworthy: (*a*) Blood serum has completely prevented digestion by the enzymes contained in the extract obtained from tuberculous tissue, whereas there has been only moderate inhibition in the presence of an equal quantity of exuded tuberculous serum; (*b*) increase of the quantity of tuberculous serum from 2.5 to five cubic centimeters has not increased the degree of inhibition, but has actually increased the amount of digestion. This fact has

been explained by the demonstration of unchecked enzyme in the exuded tuberculous serum; experiments showing this proteolytic activity of the serum will be described.

It has been shown (Experiments 1 and 2) that tuberculous tissue obtained between the second and third week after inoculation contains two enzymes: (*a*) leucoprotease of the polynuclear leucocytes and (*b*) an enzyme which digests in acid and resembles, if it is not identical with, the enzyme which we have previously designated lymphoprotease. Since experiments demonstrate partial inhibition of this combination of two enzymes, it has been considered desirable to examine the behavior of each of the two enzymes in the presence of the exuded tuberculous serum.

In order to test the effect of serum from a tuberculous pleural exudate upon leucoprotease, twenty milligrams of a dried powder prepared from cells of a sterile purulent exudate obtained by repeated intrapleural injection of turpentine have been allowed to act upon coagulated proteid in the presence of the serum to be tested.

EXPERIMENT 10.

Effect of Exuded Tuberculous Serum upon Leucoprotease.

	Control.	After 5 days at 37° C.	Digestion
20 mgr. powdered leucocytes (leucoprotease) + coag. proteid	1.95	15.9	13.95
20 mgr. powdered leucocytes + 1 c.c. exuded tuberc. ser. + coag. proteid	2.2	11.2	9.0
20 mgr. powdered leucocytes + 2 c.c. exuded tuberc. ser. + coag. proteid	2.5	10.35	7.85

Since an alkaline medium is favorable to the action of leucoprotease, but inhibits other enzymes, *e. g.*, lymphoprotease, which may be present in the exuded tuberculous serum, the effect of this serum upon leucoprotease has been tested in an alkaline medium.

One cubic centimeter of the exuded tuberculous serum has completely inhibited proteolysis by the enzyme of polynuclear leucocytes in the presence of an alkaline medium; a much greater quantity of serum causes slight digestion perhaps referable to enzyme contained in the serum (see Experiment 18). The serum of the tuberculous

exudate, like the serum of the blood, inhibits that enzyme which digests in the presence of alkali (leucoprotease).

EXPERIMENT 11.

Effect of Exuded Tuberculous Serum upon Leucoprotease in Presence of 0.2 Per Cent. Sodium Carbonate

	Control.	After 5 days at 37° C.	Digestion.
20 mgr. powdered leucocytes + coag. proteid..	3.3	10.4	7.1
20 mgr. powdered leucocytes + 0.2 c.c. exuded tuberc. ser. + coag. proteid.....	3.35	8.35	5.0
20 mgr. powdered leucocytes + 1 c.c. exuded tuberc. ser. + coag. proteid.....	3.6	3.6	0
20 mgr. powdered leucocytes + 5 c.c. exuded tuberc. ser. + coag. proteid.....	4.6	7.1	2.5

Attention has been next directed to the effect of tuberculous serum upon the enzyme which acts in acid. As it is difficult to obtain when needed for the experiment fresh tuberculous tissue containing the last-named enzyme alone (resembling that, for example, used in Experiment 3), the attempt has been made to determine the effect of tuberculous serum upon the similar, if not identical, enzyme of lymphatic glands. This experiment is less complicated than that with tuberculous tissue obtained at a time when polynuclear leucocytes are present, for the cells obtained from the lymphatic glands cause digestion in an acid and neutral medium, but produce little or no change in the presence of alkali, that is, leucoprotease is almost wholly wanting. The power of 0.1 gram of lymph gland prepared by the method previously described to cause digestion of coagulated serum has been as follows:

EXPERIMENT 12.

Normal Lymphatic Gland.

	Control.	Acetic acid, 0.2 per cent.	Neutral.	Sod. carb. 0.2 per cent.
0.1 gm. lymph. gland + coag. proteid..	2.35	7.65	5.5	3.35

The addition of small quantities of serum obtained by centrifugalization of a tuberculous pleural exudate causes increased rather than diminished digestion in an approximately neutral mixture. Serum from the tuberculous pleural exudates of two animals has been used.

EXPERIMENT 13.

Lymphatic Gland with Serum of a Tuberculous Exudate.

Control (0.1 grm. lym. gl. + coag. proteid)	2.35
	After 5 days at 37° C.
0.1 grm. lym. gl. + coag. proteid	5.5
0.1 grm. lym. gl. + 0.2 c.c. exuded tuberc. ser. No. i + coag. proteid	8.3
0.1 grm. lym. gl. + 1.0 c.c. exuded tuberc. ser. No. i + coag. proteid	12.55
0.1 grm. lym. gl. + 0.2 c.c. exuded tuberc. ser. No. ii + coag. proteid	6.75
0.1 grm. lym. gl. + 1.0 c.c. exuded tuberc. ser. No. ii + coag. proteid	8.35

Tuberculous sera No. i and No. ii have been obtained from pleural exudates withdrawn fifty days after inoculation of two animals with *B. tuberculosis.* Exudate No. i contains very few cells; no tubercle bacilli are found in a stained specimen. Fluid is alkaline to litmus. Exudate No. ii contains cells in moderate number and polynuclear leucocytes are predominant; no tubercle bacilli are found. Fluid is alkaline to litmus.

The following experiment confirms that just described.

EXPERIMENT 14.

Lymphatic Gland with Serum of a Tuberculous Exudate.

Control (0.1 grm. lym. gl. + coag. proteid)	2.1
	After 5 days at 37° C.
0.1 grm. lym. gl. + coag. proteid	4.0
0.1 grm. lym. gl. + 0.2 c.c. exuded tuberc. ser. + coag. proteid	6.25
0.1 grm. lym. gl. + 1.0 c.c. exuded tuberc. ser. + coag. proteid	11.55

Exuded tuberculous serum used in this experiment has been obtained forty-three days after inoculation from the same animal as serum No. i, Experiment 13. Cells are scant; one large mononuclear cell contains a tubercle bacillus. The fluid is alkaline to litmus.

Since the serum of the tuberculous exudate in association with enzyme of the lymphatic gland causes much more digestion than this enzyme is capable of causing alone, the possibility is suggested that the exuded serum itself may have proteolytic power.

In the following experiment measured quantities of serum obtained by centrifugalization of a tuberculous exudate have been allowed to act upon the usual quantity of coagulated proteid and at the same time comparison has been made with the digestive power of an equal quantity of fresh blood serum.

EXPERIMENT 15. The tuberculous exudate used in the following experiment has been obtained from the pleural cavity fifty-six days after inoculation with

tubercle bacilli. It is almost colorless and only slightly turbid. Microscopical examination shows that cells are present in very small numbers; a few well-preserved polynuclear leucocytes are found and mononuclear cells which are present are often vacuolated and possess poorly-stained nuclei. No tubercle bacilli are found in the stained specimen. After prolonged centrifugalization almost clear serum has been obtained and has been used for the following test.

Serum of Tuberculous Pleural Exudate.

	Control.	After 5 days at 37° C.	Digestion
0.2 c.c. exuded tuberc. ser. + coag. proteid.....	2.6	4.1	1.5
1.0 c.c. exuded tuberc. ser. + coag. proteid.....	2.9	10.95	8.05
5.0 c.c. exuded tuberc. ser. + coag. proteid.....	4.0	11.55	7.55
0.2 c.c. blood serum + coag. proteid.....	2.6	2.9	0.3
1.0 c.c. blood serum + coag. proteid.....	2.8	3.4	0.6
5.0 c.c. blood serum + coag. proteid.....	3.9	4.2	0.3

Whereas active digestion is caused by tuberculous serum digestion referable to the action of blood serum is almost wholly wanting. The results of digestion with tuberculous serum alone are in harmony with those obtained when tuberculous serum has been allowed to act in combination with enzyme from tuberculous tissue or from lymphatic gland. Digestion is not always so active as that just described (Experiment 15), but similar results have been obtained in Experiments 17 and 18 which will be described for another purpose. In the following experiment tuberculous exudate removed from the pleural cavity twelve and fourteen days after inoculation has been employed.

Experiment 16. Exudate (15 c.c.) removed from the pleural cavity twelve days after inoculation with tubercle bacilli is very turbid and contains cells in considerable number. Polynuclear leucocytes constitute about 60 per cent. of the cells present; large mononuclear leucocytes, usually collected into clumps, 38 per cent.; lymphocytes, 2 per cent. The proteolytic activity of the serum obtained by centrifugalization is as follows:

Serum of Tuberculous Pleural Exudate.

	Control.	After 5 days at 37° C.	Digestion.
1 c.c. exuded tuberc. ser. + coag. proteid.....	2.2	7.35	5.15
5 c.c. exuded tuberc. ser. + coag. proteid.....	3.25	8.9	5.65

Two days later the animal has been killed with ether. The right pleural cavity contains 30 c.c., the left 26 c.c. of turbid fluid; masses of tuberculous tissue are found in the usual situations. The exudate contains cells in the following proportions: polynuclear leucocytes, 74.5 per cent.; large mononuclear cells, 17

per cent.; lymphocytes, 8.5 per cent. One large mononuclear cell contains a tubercle bacillus. The proteolytic activity of the serum obtained by centrifugalization of the exudate has been compared with that of blood serum from the same animal.

Serum of Tuberculous Pleural Exudate Compared with Serum of Blood.

	Control.	After 5 days at 37° C.	Digestion.
0.2 c.c. exuded tuberc. ser. + coag. proteid.....	1.65	3.35	1.7
1.0 c.c. exuded tuberc. ser. + coag. proteid.....	1.8	5.6	3.8
5.0 c.c. exuded tuberc. ser. + coag. proteid.....	2.95	5.55	2.6
0.2 c.c. blood serum + coag. proteid.....	1.7	2.2	0.5
1.0 c.c. blood serum + coag. proteid.....	2.0	2.45	0.45
5.0 c.c. blood serum + coag. proteid.....	3.6	3.8	0.2

The second test made two days after the first has shown diminished proteolytic activity, perhaps as the result of the previous removal of a considerable part of the fluid in the chest; nevertheless digestion occurs, although the blood serum of the same animal is almost wholly inactive.

The experiments have shown that the serum obtained from a tuberculous pleural exudate possesses proteolytic activity which is almost wholly wanting in the serum of the blood. Absence of antienzymotic action exerted by the exuded serum allows the action of an enzyme contained in the tuberculous exudate. The behavior of this enzyme has been tested in acid, in an approximately neutral and in an alkaline medium, by allowing the same quantity of tuberculous serum to act upon coagulated proteid in the presence of various media.

EXPERIMENT 17.

Action of Exuded Tuberculous Serum in Various Media.

	Control.	Acetic acid. 0.2 per cent.	Neutral.	Sod. carb. 0.2 per cent.
1 c.c. exuded tuberc. ser. + coag. proteid.....	1.9	3.0	7.75	2.0

The serum used has been obtained from a tuberculous pleural exudate withdrawn from the peural cavity sixty-two days after inoculation.

Repetition of the experiment has given almost identical results.

EXPERIMENT 18.

Action of Exuded Tuberculous Serum in Various Media.

	Control.	Acetic acid. 0.2 per cent.	Neutral.	Sod. carb. 0.2 per cent.
2.5 c.c. exuded tuberc. ser. + coag. proteid....	2.0	4.8	10.2	3.0

The serum used has been obtained from the animal of Experiment 17 seventy-three days after inoculation.

Digestion is active in the presence of an approximately neutral medium, comparatively slight in the presence of acid and almost absent in a neutral medium. This relation to change of reaction does not accurately correspond with that exhibited by either of the two enzymes which have been found in tuberculous tissue, since one digests with almost equal activity in acid and neutral media, whereas the other digests with approximately equal activity in an alkaline and in a neutral medium.

Since the exuded tuberculous serum possesses an ability to cause proteolysis, which is absent in the serum of the blood, it is important to know if the serum of an inflammatory exudate obtained by other means possesses the same property. For the purpose of comparison an inflammatory exudate has been obtained by injecting turpentine into the pleural cavity of the dog. Samples of fluid have been removed on the third and on the fifth day after injection; the serum obtained by centrifugalization of this exudate has been employed in the following experiment:

EXPERIMENT 19.

Serum of Exudate Obtained on the Fifth Day after Injection of Turpentine.

	Control.	After 5 days at 37° C.	Digestion.
1 c.c. exuded serum + coag. proteid	2.75	4.35	1.6
5 c.c. exuded serum + coag. proteid	2.0	2.85	0.85

Serum of Exudate Obtained on the Third Day after Injection of Turpentine.

	Control.	After 5 days at 37° C.	Digestion.
1 c.c. exuded serum + coag. proteid	1.65	2.9	1.25
2.5 c.c. exuded serum + coag. proteid	2.1	3.7	1.6

Comparison of these results with digestion caused by the serum of a tuberculous exudate shows that proteolysis occurs in the presence of serum of the acute inflammatory exudate produced by turpentine, but is slight.

CONCLUSIONS.

Epithelioid cells which form the chief element of tuberculous tissue contain an enzyme which causes active digestion of proteid in an approximately neutral or in a weakly acid medium, but is inactive in the presence of weak alkali. This enzyme resembles that

which occurs in the large mononuclear cells of an inflammatory exudate and is more active than the similar enzyme of parenchymatous organs such as the liver.

The enzyme which digests in the presence of acid exhibits greatest activity at a time when caseation is beginning. With advance of caseation its activity diminishes so that tissue which has undergone almost complete caseation exhibits trivial evidence of the presence of enzyme. It is probable that complete caseation is followed by total disappearance of enzymes.

Tuberculous tissue contains an enzyme capable of digesting proteid in the presence of alkali (leucoprotease) only during the early stages of its development. This enzyme, present at a time when the tissue contains numerous polynuclear leucocytes, quickly disappears so that when enzyme digesting in acid is still active, leucoprotease has disappeared.

The serum of a tuberculous pleural exudate obtained by intrapleural inoculation with tubercle bacilli causes slight inhibition of the mixture of enzymes contained in tuberculous tissue shortly after inoculation. The serum of blood causes complete inhibition of the enzymes contained in the same tuberculous tissue. Analysis of this difference indicates that the exuded tuberculous serum, like the serum of the blood, inhibits proteolysis caused by leucoprotease, but fails to inhibit digestion caused by an enzyme acting in the presence of acid. In testing this property of the exuded tuberculous serum lymphatic gland has been used because suitable tuberculous tissue has not been available.

The serum of the tuberculous pleural exudate produced experimentally not only fails to exert the anti-enzymotic power which is exhibited by the serum of the blood, but is itself capable of causing active digestion of coagulated proteid. Normal blood serum does not digest proteid and the serum of a sterile inflammatory exudate obtained by injection of turpentine into the pleural cavity has caused very little digestion. The tests which have been made indicate that loss of anti-enzymotic power and ability to cause proteolysis increase with the age of the exudate.

The foregoing facts offer suggestions which may serve to explain in part the nature of the tubercle and the changes which occur

within it. The so-called epithelioid cells of the tubercle resemble the large mononuclear phagocytes of inflammatory exudates and both contain an enzyme of the same character. It is not improbable that caseation which, like autolysis, is accompanied by disappearance of nuclei is in part dependent upon the presence in the cells of this active proteolytic enzyme which is for a time held in check. Injury to cells by products of the tubercle bacillus or partial anæmia, the result of imperfect vascularization of the tuberculous tissue, may have a part in rendering these cells susceptible to self-digestion. Changes which have been observed in serum of the tuberculous exudate show that the anti-enzymotic property of the normal blood may be absent in the exudate of a tuberculous lesion. This loss of anti-enzymotic action, perhaps referable to changes caused by products of the tubercle bacillus, may favor self-digestion of the enzyme-containing cells and diffusion of their enzyme.

THE ENZYMES OF FIBRINOUS EXUDATES—THE EFFECT OF ONE ENZYME UPON ANOTHER.*

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In a previous study[1] I have found that fibrin of the blood contains two enzymes which are capable of acting on a foreign proteid, such as heated blood serum. Proteolysis occurs in the presence of 0.2 per cent. sodium carbonate and is inhibited by greater strengths of the alkali. Digestion also occurs in the presence of acid, though to a smaller extent, and with less constancy. Since polynuclear leucocytes have been shown to contain an enzyme which digests only in the presence of an alkaline or neutral medium, the facts just mentioned have been believed to give evidence that two enzymes exist in the fibrin of the blood, one, leucoprotease, peculiar to the polynuclear leucocytes, and a second, an enzyme which digests in the presence of acid, and is similar to the enzyme present in the large mononuclear cells of an inflammatory exudate.

It has been believed that a study of an inflammatory fibrinous exudate might better define the peculiarities of these two enzymes, and afford opportunity of studying changes which they undergo during the progress of an inflammatory reaction.

Fibrinous exudates are readily obtained by injection of turpentine into the pleural cavity.[2] After injection, fluid rapidly accumulates in the chest. This fluid is coagulable, and fibrin is deposited abundantly. Accumulation of fluid reaches a maximum in from two to four days and quickly disappears so that after six days the cavity contains no fluid. Fibrin, however, remains, gradually diminishes in amount, and in about two weeks has in most cases entirely disappeared, the cavity returning to normal. It has seemed probable that disappearance of fibrin is due to action of enzymes.

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[1] *Jour. of Exper. Med.*, 1908, x, 343.

[2] *Jour. of Exper. Med.*, 1907, ix, 391.

Exuded fibrin suspended in various media undergoes autolysis; solution, indicated by disappearance of fibrin and presence of biuret reaction after coagulation, occurs with constancy when the fibrin is incubated in an acid medium, but is less constant in neutral, or in alkaline media. In the early stages of the inflammatory reaction, after from two to four days, fibrin undergoes autolysis in the presence of sodium carbonate, but at a later period the power of digestion is completely lost. Disappearance of the fibrin of the blood when suspended in various solutions has been found an inaccurate measure of the proteolytic enzymes which it contains. A far more accurate method is to determine by use of the Kjeldahl method for nitrogen the power of a weighed quantity of the enzyme-containing substance to act on denaturalized proteid.

It has been considered advisable to apply the same method to exuded fibrin, with the purpose of determining more accurately what changes occur during the course of the inflammatory reaction. This study has shown that conditions occur, under which each of the two enzymes previously defined, exists alone, and has given opportunity to determine the character of digestion caused by combination of these enzymes.

Of the washed, pressed and shredded fibrin of the sterile exudate, 0.3 gram has been added to flasks containing ten cubic centimeters of diluted and heated blood serum, with sufficient salt solution to make a volume of twenty-five cubic centimeters, after the flasks have received the necessary amount of acetic acid, or sodium carbonate to yield the required strength. Strengths of acid above 0.2 and 0.5 per cent. have been used, with the purpose of obtaining evidence concerning the optimum medium for the action of the enzyme.

Comparison between experiments in which digestion has occurred in the presence of acid and those in which such digestion has been almost completely absent has shown two facts. First, with active digestion in presence of acid, a maximum occurs in strength of 0.2 per cent., or occasionally 0.5 per cent., and is less with greater concentration. Second, in the presence of a strength of acid greater than 1 per cent., complete coagulation of proteid by heat is difficult, so that the figures obtained are somewhat larger than is con-

sistent with entire inhibition of the enzyme. It is moreover by no means improbable that slight hydrolysis may occur in the absence of enzyme, as the result of the action of acid alone. In later experiments it was considered unnecessary to use higher strengths of acid, 0.2 and 0.5 per cent. giving a sufficiently clear indication of the enzymic contents of the fibrin tested. For the same reason, the higher strengths of carbonate were later omitted.

The following table gives the results of a systematic study of the enzymes present in fibrin removed from two to six days after a single injection of one cubic centimeter of turpentine:

TABLE I.

Experiment.	Age of Exuded Fibrin.	Control.	Acetic Acid.						Neutral.	Sodium Carbonate.				
			5.0 Per Cent.	3.0 Per Cent.	2.0 Per Cent.	1.0 Per Cent.	0.5 Per Cent.	0.2 Per Cent.		0.2 Per Cent.	0.5 Per Cent.	1.0 Per Cent.	2.0 Per Cent.	3.0 Per Cent.
1	2 day	2.6		5.30	5.50	7.25	7.95	7.15	23.50	8.35	9.00	9.20		
2		1.8					8.4	8.55	15.6	13.75	8.55			
3	3 day	2.85		5.8	5.6	6.2	7.55	8.35	13.2	3.0	3.6	3.65	5.1	4.85
4		2.4		3.95	4.3	5.1	6.8	5.9	7.5	2.8	3.1	3.7	4.25	4.2
5	4 day	1.95			5.1	6.0	7.75	9.00	8.75	2.3	2.45	3.0		
6		1.95			4.9	5.3	6.65	7.7	12.7	5.7	5.2	6.3		
7	5 day	1.85		3.85	3.15	4.8	5.8	5.5	17.05	4.0	3.3	3.75	4.2	3.4
8	6 day	2.0	5.05		3.13		8.45	9.05	17.2	4.05	4.5	4.7		

Of special significance is the fact that fibrin of two days (that is, removed at the end of two days after injection) digests with considerable activity in the alkaline media, but after this period digestion is for a time (three and four days), almost completely absent, the figures obtained in 0.2 per cent. being little greater than the control; again digestion is slightly increased at the end of four, five or six days.

In an acid medium digestion occurs in all the specimens examined. In fibrin of two days it may be assumed that at least two enzymes are present, for it has already been pointed out that the enzyme which acts in an alkaline medium can cause little if any digestion in an acid medium.[8] The enzyme which acts in an acid medium is further shown by Experiments 3, 4 and 5 to be practically inactive in the presence of alkali. Both enzymes are, however, capable of

[8] *Jour. of Exper. Med.*, 1905, vii, 316.

active proteolysis in a neutral medium, and when both are combined as in Experiments 1 and 2, digestion is far more active in a neutral medium than in either an acid or an alkaline fluid. Nevertheless the table shows that maximum activity in a neutral medium is not uniformly dependent upon combination of the two enzymes, and in only one experiment, No. 5, is digestion more active in the presence of acid than in a neutral medium. Again, after four, five and six days, with slight activity in the presence of alkali, digestion in a neutral medium far exceeds that in acid. Fibrin of three days, and one of the specimens of four days, agree in showing an almost complete absence of digestion in the presence of sodium carbonate, yet their behavior in neutral and acid media is not constant, for whereas digestion in fibrin of three days is far more active in a neutral medium, in that of four days (Experiment 5) digestion is more active in the presence of acid. This fact has remained unexplained.

The two members of each group, containing animals killed after two, three and four days respectively, have been so arranged within the individual group that there is at first a continuous decrease and later a continuous increase of digestion in a neutral medium. Such an arrangement shows that digestion in the neutral medium which is very active at the end of two days, diminishes to a minimum between three and four days, and again gradually increases after the fourth day.

In the foregoing table, fibrin has been obtained from sero-fibrinous exudates produced by a single injection of turpentine. In a study of experimental pleurisy,[4] Dr. Opie has found that repeated injections of turpentine cause continued emigration of polynuclear leucocytes which give the fibrin increased power of autolysis in the presence of alkali. The fibrin becomes more succulent and softer. The serous exudate becomes more turbid and finally assumes the appearance of pus. Such fibrin, unlike that previously employed, when suspended in an alkaline medium has been found to undergo very active autolysis. The Kjeldahl method used in the foregoing experiments has been employed to determine the power of a weighed quantity of this fibrin to cause digestion of coagulated serum. The following table represents the digestion of coagulated blood serum

[4] *Jour. of Exper. Med.*, 1907, ix, 415.

under conditions analogous to those of the experiments recorded in Table I.

TABLE II.

Experiment.	No. of Days After First Injection of Turpentine.	Control.	Acetic Acid.						Neutral.	Sodium Carbonate.		
			5.0 Per Cent.	3.5 Per Cent.	2.0 Per Cent.	1.0 Per Cent.	0.5 Per Cent.	0.2 Per Cent.		0.2 Per Cent.	0.5 Per Cent.	1.0 Per Cent.
9	5	2.8				4.25	4.7	4.45	21.00	21.5	9.85	8.9
10	5	3.2	4.5	4.35	4.45		3.7	3.25	16.1	17.55		7.9

Comparison of Tables I and II shows that great increase in leucoprotease, as the result of a second injection of the inflammatory irritant, has been associated with almost complete disappearance of ability to digest in an acid medium. In both experiments of Table II, the second injection of turpentine has been received three days after the first and the fibrin removed from the chest two days later; comparison with Table I shows that the enzyme digesting in acid and present during this period may be completely replaced, perhaps destroyed by leucoprotease.

Experiments 3, 4 and 5 of Table I are examples of digestion caused by that ferment which acts in acid and is inhibited by alkali; digestion in alkali is almost wholly absent. The two experiments of Table II on the contrary exhibit the action of that ferment which acts in the presence of alkali and is inhibited by acid. Especially noteworthy is the fact that both enzymes are capable of acting in an approximately neutral medium. This fact best explains their action in the body. Each is capable of exerting an almost maximum activity in a neutral medium, whereas a slight change of reaction favors one and inhibits the other.

Should an exudate contain both enzymes, the method which has been employed would demonstrate proteolysis in both acid and alkali. In a neutral medium the two enzymes acting simultaneously might cause far greater digestion. Such combination of enzymes with maximum activity in the neutral medium is illustrated by Experiments 1, 2, 6, 7 and 8. In the following experiment the simultaneous occurrence of two enzymes has been demonstrated by using a method which previous experiments have shown preserves one enzyme and injures the other. After three injections of turpentine

into the right pleural cavity, fibrinous exudate has been obtained in great part from the left cavity. This fibrin has been tested in the fresh state under the usual conditions; a part has been treated with alcohol and ether, dried and powdered and a weighed quantity approximately corresponding to the usual amount of fresh fibrin has been used. It has been previously found that this method preserves almost exclusively leucoprotease and fails to preserve the enzyme which has been designated as lymphoprotease.[5]

TABLE III.

Experiment.	Amount of Fibrin Used.	Control.	Acetic Acid.					Neutral.	Sodium Carbonate.		
			3.0 Per Cent.	2.0 Per Cent.	1.0 Per Cent.	0.5 Per Cent	0.2 Per Cent.		0.2 Per Cent.	0.5 Per Cent.	1.0 Per Cent.
11	0.3 gms. fresh.	2.25	3.85	4.2	6.05	6.5	7.4	19.35	8.05	6.4	6.85
12	20 mgr. powd.	2.7	4.05	3.6	4.25	4.55	4.7	20.05	14.35	5.3	6.7

The experiment shows that drying of fibrin has markedly diminished its activity in the presence of acid, and at the same time has increased its activity in the presence of alkali (0.2 per cent. sodium carbonate). Digestion in a neutral medium has remained almost unimpaired. Table II on the one hand gives evidence that leucoprotease may destroy the enzyme which acts in acid (lymphoprotease); Table III on the other suggests that the last-named enzyme may under certain conditions destroy or inhibit leucoprotease, since removal of its influence increases the activity of digestion in alkali.

CONCLUSIONS.

By a study of the enzymes contained in fibrinous exudates produced by injection of a sterile inflammatory irritant (turpentine) conditions have been found in which each of two enzymes occurs alone. The one, leucoprotease, digests in the presence of alkali; the other, resembling lymphoprotease, digests in the presence of acid, yet both exhibit almost maximum activity in an approximately neutral medium. It is probable that both enzymes, in the body, exert their greatest activity in an approximately neutral medium, slight changes in reaction increasing digestion by the one, and suspending digestion by the other.

[5] *Jour. of Exper. Med.*, 1907, ix, 415.

The enzyme digesting in acid, present in the fibrinous exudate obtained after a single injection of turpentine, disappears when repeated injection of the same irritant transforms the sero-fibrinous into a purulent exudate, and causes accumulation of leucoprotease in great quantity.

The assistance and oversight given by Dr. Opie in the present study are gratefully acknowledged.

ON COMPLEMENT-FIXATION IN MALIGNANT DISEASE.[1]

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Through the researches of Wassermann and his collaborators it has been established that syphilitic sera may contain substances which in the presence of other substances derived from syphilitic organs are capable of fixing complement, so that upon the subsequent addition of red corpuscles and homologous hemolytic amboceptor hemolysis is impeded. This phenomenon was originally interpreted as meaning that as a consequence of the syphilitic infection specific antibodies are formed which enter into combination with the corresponding antigen—present in syphilitic organs—and that the resultant product is capable of binding complement. Subsequent study has shown that this conception does not satisfactorily account for the facts observed since similar fixation of complement on the part of syphilitic serum may occur in the presence of non-syphilitic tissue extracts. While a different interpretation of the phenomenon must accordingly be sought, the fact remains that substances are formed in the body of syphilitic individuals which will react with certain tissue constituents and bind complement and that the reaction may in a certain measure be regarded as specific. Wassermann states in a recent communication that approximately a thousand cases have been examined, up to the end of the previous year, from which the diagnostic value of the reaction (ergo, its specificity) is uniformly apparent.

Our own work had already been planned at a time when the antigen-antibody interpretation of the syphilitic reaction had not yet been set aside, and when it seemed that the same principle might well be utilized in a search for antibodies in malignant disease. The applicability of the method in the study of pathological conditions, in which the causative agent is unknown, had indeed

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been already suggested by Wassermann. At the very outset we were, of course, confronted with the rapidly accumulating facts which go to show that the cancer cell itself may be the parasite proper in malignant disease, and the question naturally suggested itself whether it would be likely under such conditions that any results would be attained.

It seemed that specific antibodies could only be expected, if a specific antigen were operative, and the idea of a specific antigen at first thought appeared likely only if a specific cancer parasite could be assumed to exist. But on further consideration it seemed quite possible that even in the absence of an extraneous factor the cancer cell itself might either give rise to products qualitatively different from those of its normal antecedents, or that, as a result of increased cellular destruction, normal cell products might appear in largely increased amount, and give rise to corresponding antibodies. In either event the cancer cell could be viewed as the antigen and the resultant antibodies would accordingly be auto-antibodies. That auto-antibodies may actually be formed seems to be a well-established fact. It is thus known that auto-hemolysins appear in the serum following the resorption of extravasations of blood. Centanni[2] has demonstrated the appearance of auto-hepato-precipitins in sheep and cattle distomiasis. Bergmann and Salvini[3] have rendered it probable that auto-antibodies are formed in phosphorus poisoning. Schütze and Ascoli[4] succeeded in obtaining auto-precipitins through the injection of homologous albumins, and some observers indeed explain the Wassermann reaction in syphilis upon the basis of auto-antibody formation.[5] The idea that corresponding substances may be formed in malignant disease would accordingly not be far-fetched, and as a matter of fact we have demonstrated that this may actually occur. This

[2] Centanni, E. Contributo alle autocitoreazioni: precipitina e sottrazione del complemento. *Policlinico,* 1906, xiii, 840.—*Idem,* Sulle autocitoprecipitine. *Atti della Societa Italiana di Patologia,* 1906, 404; cited *Jahresbericht über d. Ergebnisse der Immunnitätsforschung,* 1908, ii, 113.

[3] v. Bergmann, S., and Salvini, F. Das hæmolytische Hemmungsphenomen b. Phosphorvergiftung u. anderen pathologischen Prozessen. *Zeit. f. exper. Pathol. u. Therap.,* 1907, iv, 816.

[4] Schütze and Ascoli, cited by Weil, E., and Braun, H. See Note 5.

[5] Weil, E., and Braun, H., Uber Anti-körperbefunde bei Lues, Tabes u. Paralyse. *Berl. klin. Woch.,* 1907, xliv, 1570.

conclusion is warrantable at least upon the basis that the complement fixation method is applicable to decide the question at issue.

TECHNIQUE.

Preparation of the Antigen.—We are aware of the fact that no definite proof has as yet been afforded to show that the reaction is based upon the antigen-antibody principle, and we use the term antigen in this connection merely as a matter of convenience, and to designate in a general way the substance which in the presence of a certain reaction product of the blood serum is capable of fixing complement. Leaving out of consideration, for the present, the question of a specific extraneous cancer parasite, and viewing the cancer cell itself as the essential offending element, which in some manner as yet unknown, gives rise to the formation of corresponding reaction products, it seemed advisable to prepare antigens from different sources and to choose the homologous product in the examination of the individual case, viz., to place the blood serum from a case of cancer of the breast into reaction with breast cancer antigen, the blood serum from a cancer of the stomach with stomach cancer antigen, etc. Upon further consideration, however, we came to the conclusion that it would be wiser at this stage of our research to make use only of tumors in which bacterial infection could be eliminated. Working with extracts from gastro-intestinal cancers it was practically a foregone conclusion that our antigens would also contain large numbers of bacteria, that in the corresponding cases bacteriolysin formation would have taken place, and that any hemolytic inhibition that might be observed could not be attributed exclusively to a cancer reaction. We accordingly made use only of breast cancers, but found that it was necessary to eliminate those in which connective tissue formation stood in the foreground. The common scirrhus is altogether unsuitable as antigen. The best results are obtained with medullary cancers. The tumor material was always obtained at operation, the cancerous portions dissected out, freed from fat as far as possible and ground up with glass in a mortar into a thick paste. This was placed in a suitable receptacle and shaken for about twenty-four hours in a shaking machine with an amount of one-half per cent. carbolic acid

in normal salt solution, sufficient to make an emulsion of moderate consistence. A small amount of thymol was further added as preservative, as the half per cent. carbolic acid is not sufficient to prevent putrefactive changes. The resultant material was kept in the ice-box without removing the saline extract from the tissue. Prolonged exposure to light seems to destroy the activity of the reacting substance, but preserved at low temperature in the dark, we could not detect any loss in strength during a period of at least two months. From the concentrated extract our antigen was freshly prepared on each occasion by contrifugalizing a small amount for at least one hour, at high speed and diluting the supernatant fluid with normal salt solution to a point where no inhibitory effect could be obtained in the presence of the usual amount of *normal* blood serum and fresh guinea-pig complement. This point must be carefully determined, as more concentrated emulsions will by themselves fix complement to an extent that hemolysis may be entirely prevented upon the subsequent addition of the hemolytic amboceptor and corpuscles. The diluted antigen, when ready for use, will of course also fix a certain amount of complement, but the degree of dilution must be so chosen that with normal serum and fresh complement no inhibition of hemolysis will be noticeable after remaining in the incubator for one hour and a half. A determination of the albuminous content of our ultimate dilutions showed about 0.4 per cent. Every antigen before use must, of course, be tested with an appropriate serum, in order to demonstrate that it is capable of inhibiting hemolysis in the presence of such serum (see also below).

The Patient's Serum.—In our work it was not practical for various reasons to obtain the patient's blood from the vein, but we found it perfectly convenient to milk the necessary amount from the ear, after a free puncture with a small lancet or a Hagedorn needle; in some instances larger quantities were obtained at the time of the operation. As the amount of blood at our disposal was smaller than is usually demanded in work of this character we were obliged to make use of smaller quantities also in charging our tubes—0.5 cubic centimeter instead of one cubic centimeter of the diluted serum being our standard amount. This was en-

tirely sufficient, and we would emphasize the value of this method of procuring the blood over venous puncture, especially when many specimens of blood must be procured at one time, and when repeated examinations are to be made. As we were naturally somewhat restricted in the amount of serum, however, in our experiments we worked with single five-fold dilutions of the serum only, tests with diminishing amounts being neglected, and for our purposes unnecessary.

In by far the larger number of our experiments the blood serum was examined within a few hours after the blood was drawn, as Wassermann and his pupils have pointed out that in the syphilitic examinations certain sera may alter their behavior materially on standing, both toward syphilitic and normal tissue extracts. We found, however, that our sera after inactivation for 30 minutes at 52° C. retained their specific activity toward cancer antigen for a number of weeks without impairment whatsoever. We have on hand at present a specimen which was secured seven weeks ago and is as active to-day as when first drawn.

If for any reason our sera could not be examined on the day on which they were obtained, they were separated from the corpuscles as soon as possible; inactivated and then kept, without any preservative, in tightly stoppered tubes in the refrigerator. In several instances carbolic acid was added to the extent of one-half per cent., but we abandoned this, as it appeared that this amount, even after subsequent dilution caused some complement fixation, (sc. destruction).

Our *hemolytic system* consisted of hen corpuscles, anti-hen-rabbit serum, and guinea-pig complement.

The use of hen corpuscles was suggested to us by Dr. R. V. Lamar of the Rockefeller Institute for Medical Research and proved very convenient, as the finer grades of complement fixation can be more readily recognized from the "Schimmer" of the intact corpuscles, which is due no doubt to their form and nucleation. A five per cent. emulsion was employed.

The hemolytic amboceptor was of such strength that .002 cubic centimeter would cause the complete hemolysis of one cubic centimeter of the corpuscle emulsion in the presence of 0.1 cubic centimeter of fresh guinea-pig complement.

The necessity of using fresh guinea-pig complement in our work was really one of our chief difficulties. We frequently attempted its preservation for several days in the frozen state, but found that with our facilities this was almost impossible; even when it had been so kept it happened repeatedly that, although its strength was still sufficient to hemolyze the corpuscles in the presence of hemolytic amboceptor alone, the amount of active substance was so small that the combination antigen-normal serum would absorb nearly the entire amount. We found that it was not safe to use any complement that had been kept longer than twenty-four hours. The neglect of this rule caused the loss of many days' work. We were accordingly forced to sacrifice a new guinea-pig after every two days' work. Puncture of the heart was not practical for our purposes, as we required larger amounts of blood than can be obtained in this manner. Experiments with other sera, as complement, showed that for our system of hen corpuscles and anti-hen-rabbit serum guinea-pig serum alone was applicable. Hog serum, as well as rabbit serum, causes more or less hemolysis of hen corpuscles *per se*. Human serum, chicken serum, and sheep serum are free from this objection, but apparently not rich enough in complement. With human complement particularly, even when combined with normal inactivated serum only, in the absence of any antigen, no hemolysis whatsoever may occur. This in itself is a very interesting phenomenon and would merit more extensive investigation as it seems to throw some light upon the inhibitory reaction which is observed in various pathological conditions, in the absence of any antigen.

The Individual Experiment.—The arrangement of the individual experiment with the necessary controls is apparent from the following schema:

I. *Controls of hemolytic system.*

	Diluted Antigen.	Pts. Serum Dil. 1 : 5.	Normal Serum Dil. 1 : 5.	Complement Dil. 1 : 10.	Amboceptor Dil. 1 : 500.	Corpuscles 5% Emulsion.
1	—	—	—	—	—	0.5
2	—	—	—	—	0.5	0.5
3	—	—	—	0.5	—	0.5
4	—	—	—	0.5	0.5	0.5

			II. *Antigen controls.*			
5	0.5	—	—	—	—	0.5
6	0.5	—	—	0.5	0.5	0.5
			III. *Serum controls.*			
7	—	0.5	—	—	—	0.5
8	—	0.5	—	0.5	0.5	0.5
9	—	—	0.5	—	—	0.5
10	—	—	0.5	0.5	0.5	0.3
			IV. *Serum-antigen controls.*			
11	0.5	0.5	—	—	0.5	0.5
12	0.5	0.5	—	0.5	—	0.5
13	0.5	—	0.5	0.5	0.5	0.5
14	0.5	—	0.5	—	0.5	0.5
15	0.5	—	0.5	0.5	—	0.5
			V. *Experiment proper.*			
16	0.5	0.5	—	0.5	0.5	0.5

The tubes which contain antigen-complement, serum-complement, or antigen-serum and antigen-serum-complement are incubated for one hour at 37° C. before the corpuscles and the hemolytic amboceptor are added; when two components only are combined one volume of normal salt solution (0.85 per cent.) is added and when one only is employed two volumes of saline solution are used. After the addition of the hemolytic system (viz., amboceptor and corpuscles) the tubes are returned to the incubator, the corpuscles shaken up at intervals of about fifteen minutes, and careful note taken of the progress of the hemolysis.

The most important controls, of course, in judging the progress of the hemolysis, are Numbers 8 and 13; these, besides Numbers 4, 6 and 10 should invariably be made. The others can be neglected in future cancer work, as our studies have shown that they are unnecessary, if the technique here advocated is followed; if any deviations, however, are contemplated they should all be carefully considered.

Wassermann originally advocated that the tubes, after the addition of the hemolytic system, be left in the incubator for two hours, that they should then be removed to the ice-chest and examined the following day. This seems to us entirely too arbitrary, considering the fact that we are working with several factors of

which two at least are inconstant quantities, viz., the patient's serum and the complement content of the guinea-pig serum, which certainly differs more or less in different animals. We would merely recall the loss of complement which occurs during the process of fasting. Neglect of this factor on one occasion caused us the loss of a whole day's work. We trust that ere long we may be in a position to control quantitatively the exact amount of complement that is added, but until this can be done we are scarcely justified in setting an arbitrary time limit, during which the specimens are to be kept in the incubator. There are sera, it is true, which in the presence of suitable antigen bind complement in so large a quantity that one could safely leave the tubes in the incubator for many hours without the occurrence of any hemolysis whatever, but it is more common to meet with smaller amounts of complementophilic substance, in which hemolysis is merely delayed, as compared with the controls 8 and 13. In order to demonstrate this delayed hemolysis, which, of course, must be interpreted as a certain grade of fixation, it is absolutely necessary to observe the specimens from time to time in the incubator and to regard the experiment as ended, for a given specimen, as soon as the controls show that hemolysis in these is complete. In many cases this point is reached after an hour or an hour and a quarter.

The thought naturally suggested itself that the existence of a moderate increase of complement fixation beyond normal could be demonstrated more readily, if smaller amounts of complement were used. This, however, we found impractical as the degree of complement fixation on the part of the normal sera is at times so great that the upper limit of the normal and the lower limit of the abnormal were thus brought fairly close together. To be sure, the lower limit of the abnormal seems to be even then so far above the normal, that it is possible to demonstrate the increase in most cases by the size of the "Kuppe" in the centrifugated specimen, or by the hemoglobin content of the supernatant fluid, as determined with Fleischl's haemometer, for instance. But our technical difficulties were sufficiently numerous without additional complications, so that we were content at this stage of our research to confine our attention to gross reactions and to abandon the

attempt to obtain a maximal "Ausbeute" of positive findings. For this reason especially did we continue the use of the larger amounts of complement, and attempt to overcome the resultant difficulties by disregard of an arbitrary time limit. That Wassermann and his collaborators have since come to a similar conclusion in their syphilitic work is clear from the paper of Meier,[6] and suggests itself as natural to anyone who has worked along these lines. The tubes were accordingly examined from time to time and the experiment interrupted as soon as the controls 8 and 13 showed complete hemolysis. In the majority of our cases no difficulty was experienced in deciding whether fixation had occurred or not; in some instances there was no trace of hemolysis, but as a rule partial fixation only was obtained in the positive cases; if any doubt was felt whether there was any fixation at all the specimens were centrifugalized, when the question was readily settled.

RESULTS.

While many more cases were examined than are included in the accompanying tables we have taken special pains to eliminate every observation concerning which the least doubt was felt. The list hence includes only those cases in which a well pronounced reaction was either present or absent, viz., those in which it was perfectly clear that complement fixation had or had not occurred.

A study of the tables shows at once that in the malignant cases fixation occurs quite frequently, while in the non-malignant cases it is rare. In our list which includes the most diverse pathological conditions it was indeed observed but twice and in these the fixation finds a probable explanation in the history of the patient. No. 49 was admitted to the hospital with advanced tertiary syphilis (necrosis of the turbinates and septum) and we are strongly inclined to attribute the positive reaction to this condition, since Weil and Braun have shown that the syphilitic reaction may also be obtained, if tumor extracts are used as antigen instead of extracts from syphilitic livers. In the second case, No. 42, a young colored

[6] Meier, G. Die Technik, etc., der Wassermannschen Reaction auf Syphilis. *Berl. klin. Woch.*, 1907, xliv, 1636.

TABLE I.

NON-MALIGNANT CASES.

1. G. G.	Normal	Complete	hemolysis.
2. Mr. H.	Gallstones	"	"
3. Mr. Z.	Gallstones	"	"
4. Mrs. G.	Calcified fibroid	"	"
5. Mr. J.	Empyema	"	"
6. Mr. N.	Gangrene of the foot (diabetic)	"	"
7. Mr. S.	Renal calculus	"	"
8. Mr. McL.	Chronic cystitis, nephritis	"	"
9. Mrs. St.	Benign tumor of parotid	"	"
10. Mrs. B.	Hysteria	"	"
11. Mr. K.	Tubercular sinus of the hip	"	"
12. Mr. X.	Amputation at the wrist	"	"
13. Mr. X.	Gonorrhœal arthritis	"	"
14. Mr. W.	Tuberculosis of the hip joint	"	"
15. Mr. Y.	Frost bitten feet	"	"
16. Mr. B.	Typhoid fever, convalescent	"	"
17. Mrs. P.	Septicæmia following abortion	"	"
18. Mr. W.	Erysipelas	"	"
19. Mrs. St.	Renal tuberculosis	"	"
20. Mrs. W.	Appendicitis and peritonitis	"	"
21. Mrs. E.	Suppurating appendicitis	"	"
22. Mr. X.	Uræmia	"	"
23. Mr. X.	Perihepatic abscess	"	"
24. Mr. G.	Suppurating appendicitis and perinephritic abscess	"	"
25. Mrs. N.	Inflammatory mass in pelvis	"	"
26. Mr. McG.	Hepatic cirrhosis and chronic pancreatitis	"	"
27. Mrs. B.	Suppurating tubercular case	"	"
28. Mr. H.	Cardiac lesion	"	"
29. Mr. G.	Hysteria	"	"
30. Mr. P.	Tubercular sinus	"	"
31. Mr. A.	Typhoid fever	"	"
32. Mrs. L.	Cyst of the broad ligament	"	"
33. Mrs. G.	Ovarian cyst	"	"
34. Mr. A.	Gonorrhœal rheumatism	"	"
35. D.	Compound fracture of radius	"	"
36. E.	Pneumonia, a few days after crisis	"	"
37. M.	Fracture of malar bone	"	"
38. T.	Pleurisy with effusion	"	"
39. W. T.	Normal	"	"
40. Mrs. S.	Normal	"	"
41. C. E. S.	Normal	"	"
42. L. C.	Tubo-ovarian abscess (fixation before operation); after operation	"	"
43. Mr. R.	Myeloid myelocytic leukemia	"	"

44.	Mrs. F.	Normal	Complete hemolysis.
45.	M. W.	Procidentia of the uterus	" "
46.	Miss B.	Pneumonia (convalescent)	" "
47.	Mrs. K.	Pseudoleukemia	" "
48.	Mr. St.	Appendicular abscess	" "
49.	F.	Alcoholism	Fixation.
50.		Pooled serum of four normal persons	Complete hemolysis.

TABLE II.

MALIGNANT CASES.

1.	Mrs. H.	Carcinoma of the liver	Fixation.
2.	Miss T.	Carcinoma of the rectum, p. o.	"
3.	Mrs. R.	Carcinoma of the uterus, p. o.	"
4.	Mrs. C.	Carcinoma of the breast, p. o.	"
5.	Mrs. O.	Carcinoma of the uterus, p. o.	Hemolysis.
6.	Mrs. R.	Carcinoma of the uterus, p. o.	"
7.	Mrs. F.	Carcinoma of the uterus, p. o.	"
8.	Mrs. B.	Carcinoma of the breast, p. o.	Fixation.
9.	Mr. X.	Carcinoma of the prostate, p. o.	"
10.	Mrs. S.	Carcinoma of the breast, p. o.	"
11.	Mr. K.	Malignant growth of the lower jaw	"
12.	Mrs. P.	Recurrent tumor of the parotid, a. o.	"
13.	Mrs. B.	Carcinoma of the rectum, p. o.	"
14.	Mrs. H.	Small scirrhus of the breast, p. o.	Hemolysis.
15.	Mr. G.	Recurrent carcinoma of the thyroid, a. o.	"
16.	Mrs. L.	Carcinoma of the breast, p. o.	Fixation.
17.	Mr. C.	Melanotic sarcoma	Hemolysis.
18.	Mr. D.	Carcinoma of the stomach	"
19.	Mrs. D.	Carcinoma of the breast	Fixation.
20.	Mr. W.	Carcinoma of the pancreas	Hemolysis.
21.	Mr. W.	Carcinoma(?) of the submaxilary gland	"
22.	Mr. H.	Carcinoma of the stomach	"
23.	Mr. H.	Carcinoma of the stomach	Fixation.
24.	Mr. F.	Carcinoma of the common duct and gangrenous cholecystitis	"
25.	Mrs. F.	Carcinoma of the uterus, p. o.	"
26.	Mrs. C.	Carcinomatous degeneration of a uterine fibroid	" "
27.	J. R.	Carcinoma of the stomach (?) (absence of HCl, presence of lactic acid)	"
28.	Mrs. B.	Carcinoma of the rectum, p. o.	"
29.	A. B.	Carcinoma of the ovary, p. o.	"
30.	Mr. K.	Carcinoma of the prostate, p. o.	"
31.	Miss H.	Carcinoma of the breast (recurrent), p. o.	Hemolysis.
32.	L.	Carcinoma of the stomach	"
33.	Mr. H.	Carcinoma of the stomach	"
34.	Mrs. B.	Carcinoma of the breast, p. o.	Fixation.

35. Mr. K.	Carcinoma of the œsophagus.....	Fixation.
36. Mrs. W.	Carcinoma of the uterus, p. o.	"
37. L. P.	Carcinoma of the stomach (?).....	"

p. o. indicates post operative; a. o. before operation.

woman, a direct syphilitic history could not be obtained, but the fact that she was operated for pus tubes suggested that the possibility of a preceding syphilitic infection can at least not be denied. Unfortunately we did not have appropriate syphilitic antigen at hand to test this question at the time.

It might of course be argued that since cancer antigen may cause complement fixation with syphilitic serum that some of our positive findings in the malignant column also may have been due to syphilis and not referable to the existence of malignant disease.

If then we eliminate the known case of syphilis from the non-malignant column and allow the doubtful one to remain we find that with cancer antigen no fixation occurred in 98 per cent. of all cases, while among the cancer cases 65 per cent. gave a positive reaction.

Considering the fact that we have thus far worked only with breast cancer antigen, and that the method is as yet of necessity imperfect, it must seem not unreasonable to suppose that with improved technique a still larger yield of positive findings may be obtainable, and that the reaction may become an important one in diagnosis. While we are aware of the fact that the number of cases which we have had occasion to examine is still far too small to permit any far reaching conclusions, we feel strongly tempted to state even now that barring the occurrence of syphilis a positive reaction with cancer antigen may be regarded as strong evidence of the existence of malignant disease. Our blood specimens came to us frequently, merely numbered, or labelled with the name of the patient and without any clue regarding the nature of the disease; still we were frequently able to make the diagnosis without having any previous knowledge of the patient's condition whatsoever. As an example we would cite the following instance: We have pointed out before that while in the majority of cases the complement fixation is only partial it may at times be complete. This complete reaction is so striking that it naturally attracts imme-

diate attention. We were accordingly much interested to find this in a specimen of blood which came to us with the patient's name, but without further comment. Upon inquiry whether the case were a malignant one we were told that the patient had only been admitted that morning, that there was a "lump" connected with the lower jaw, but that no further data had been obtained. The history then showed the following:

The patient was a man, æt. 70, whose general health had been excellent; there was in particular no history of syphilis; his wife had died of typhoid fever and there were six children living and in good health. About a week before Christmas, 1907 (ten weeks before admission) he first noticed a swelling in the right lower jaw which gave rise to much pain, and became rapidly larger. Upon admission to the service of Dr. Wm. Fisher, its size was about that of a small egg, over which the skin was partly adherent. It was irregular in form and extended along the floor of the mouth in the region of the submaxillary gland. Its outline was indefinite, but seemed partly connected with the bone; it was hard to the touch, decidedly tender on deep palpation, and not connected with any teeth, which had all been removed on that side many years ago. The tumor was removed by Dr. Fisher together with the ramus of the jaw and the submaxillary gland, and submitted to Dr. Bloodgood for examination, who reported that the diagnosis lay between alveolar sarcoma, endothelioma and carcinoma.

This case is particularly interesting, as the possibility exists that it is a connective tissue tumor and that the reaction was obtained with breast cancer antigen. Our list includes two other tumors of this order, Nos. 26 and 29, viz., a sarcomatous fibroid and an ovarian sarcoma.

We realize that it would be unwarrantable to draw any far-going inferences from these observations, but the finding is especially interesting since it has been shown that mice which have been rendered immune to carcinoma are also immune to sarcoma, in other words, a much closer relation must exist between the connective tissue tumors and epithelial growths than was formerly supposed.

It will be noted from a survey of our cases that in many the examination was made after operation, the interval varying between a day and several weeks. The question accordingly arises whether some of our negative results may not be owing to the fact that the blood was taken relatively late after the extirpation of the growth. While this possibility cannot be eliminated without

further investigation we surmise from our observations that the time element, within certain limitations, is not responsible for the negative findings, for we have been able to ascertain that the "inhibitory" substance does not necessarily disappear from the blood even after extensive excision, and we have even gained the impression that the amount does not necessarily diminish. In some instances, it is true, the reaction becomes materially less marked after a number of weeks and may even disappear, but in others, after a temporary decrease in its intensity, it was later found as marked as in the beginning. In Case 11, for example, about three weeks after operation, the reaction was just as intense as the day preceding the removal of the growth. How long it may remain present after operation future studies will have to show.[7]

We are thus not inclined to attribute our negative findings to the removal of the growth, but rather believe that the reaction was absent also before operation, and on considering the location of the growths in the negative cases the thought suggests itself that in these auto-inoculation with the primary degenerative products of the tumor may not occur so readily as in the others. For it will be noted that the majority of these negative cases are growths which are connected with the channels of the body which lead directly to the exterior, viz., the gastro-intestinal canal and the vagina. This question, however, cannot be decided until a much larger amount of material has been studied and classified. We mention the matter merely as a possibility. Apparently opposed to this explanation is the fact that continued vaccination of patients with shake extracts of cancer tissue does not necessarily cause the reaction to appear, when it has been absent before. Our observations, however, here also, are still too small in number to allow us to speak definitely, and the time during which the injections have been continued too short. We hope to report on this point in detail on a future occasion.

It would of course be tempting to theorize on the basis of our observations, but we purposely refrain from doing so, and are content in merely stating the facts, which have been obtained. The en-

[7] A recent examination of this case, about eight weeks after operation, showed only a very faint reaction.

tire question of complement fixation as the result of an "antigen-antibody" reaction is hardly yet open to intelligent speculation, and we would emphasize once more that we have used the term antigen and antibody in the present paper merely as a matter of convenience and do not wish to imply in the least that we regard the reacting substance in the blood serum as the expression of a defensive reaction on the part of the body, and hence as a measure of cancer immunity. We incline to the view, however, that the serum reaction product is "tuned" more or less specifically to the cancer antigen and would emphasize that negative reactions only, with cancer antigen, have been obtained in the numerous cases in which "normal" body cells were undergoing degeneration even in large numbers, as exemplified more specifically by the negative findings in the myeloid myelocytic leukemia, and in the various types of abscess formation, as noted in our first table.

While the complement fixation to which reference has been had in the foregoing pages depends upon the interaction between two factors, of which one is present in the blood serum of the patient and the other in the cancer extract, we have met with a second type, in which the inactivated serum binds complement *per se*. In our series of nearly one hundred cases this occurred but four times, viz., in two cases of hepatic cirrhosis, in one of Banti's disease, and in a patient who had passed through three operations for cancer of the breast and had been treated for six months with injections of a cancer vaccine. This phenomenon was first observed by Neisser and Doering[8] in 1901 in a case of impending uremia and has since been studied by Neisser and Friedemann,[9] Laqueur,[10] Hedinger,[11] Wolze,[12] Senator,[13] Lüdke,[14] and especially by v. Berg-

[8] Neisser and Doering, Zur Kentniss d. hæmolytischen Eigenschaften d. menschlichen Serums. *Berl. klin. Woch.*, 1901, xxxviii, 593.

[9] Neisser and Friedemann, Uber Amboceptoidwirkung in einem menschlichen Serum, *ibid.*, 1902, xxxix, 677.

[10] Laqueur, Zur Kentniss uræmischer Zustände. *Deutsch. med. Woch.*, 1901, xxvii, 744.

[11] Hedinger, Klin. Beiträge z. Frage d. Hæmolyse. *Deutsch. Arch. f. klin. Med.*, 1902, lxxiv, 24.

[12] Wolze, Zur Hemmung d. Hæmolyse b. uræmischen Zuständen. *Cent. f. inn. Med.*, 1903, xxiv, 649.

mann and Keuthe.[15] It has been met with in isolated cases of pneumonia, sepsis, carcinoma, leukemia, uremia, etc., and has been experimentally produced by v. Bergmann and Salvini in phosphorus poisoning, and by Eva Hoffman[16] in uremia and nephritis.

Since Neisser and Friedmann found a distinct decrease in the amboceptor content of the heated serum they attempted an explanation of the phenomenon by assuming the formation of amboceptoids and their union with the haptophoric groups of the red corpuscles. V. Bergmann and Keuthe, however, showed that hemolytic inhibition occurred also, if previous to heating, the amboceptors of the serum are removed by uniting them with red corpuscles and centrifugalizing, after absorption of complement by means of yeast. They accordingly conclude that hemolytic inhibition is referable to preëxistent anticomplement, which in active serum is bound by a certain amount of complement, and that as the result of heating to 56° C. the binding complement is destroyed and the anti-complement liberated. From their experimental work in phosphorus poisoning v. Bergmann and Salvini are further led to assume that in the degenerating liver substances are liberated that lead to the production of antibodies. If then both antigen and antibody are simultaneously present in the circulation, in considerable amount, the result is an impoverishment of complement, which can be directly demonstrated. If the combination antigen-antibody is present in smaller quantity the inhibitory effect may still be demonstrable in the *inactivated* serum and may be increased by the further addition of antigen. V. Bergmann and Salvini finally suggest that it might be possible to demonstrate the inhibition by the addition of a suitable amount of antigen even in cases in which, without this, hemolysis must proceed unimpeded. This explanation it

[15] Senator, Uber d. hæmolytische Eigenschaft d. Blutserums b. Uræmie. *Berl. klin. Woch.*, 1904, xli, 181.

[16] Lüdke, Beit. z. Studium d. Complemente, *Münch. med. Woch.*, 1905, liii, 2065 and 2126.

[15] v. Bergmann and Keuthe, Die Hemmung d. Hæmolyse durch inactivirte menschliche Sera, *Zeit. f. exper. Pathol. u. Therap.*, 1906, iii, 225.

[16] Hoffmann, Experimentelle Untersuchungen über d. hemmende Wirkung inactivirter Sera., *ibid.*, 704.

seems to us, may be applicable also in certain cases of cancer, as well as in the syphilitic cases and certainly deserves careful consideration. But it does not satisfactorily explain the continued inhibition after operation, unless we assume that its persistence is an indication that the cancerous tissue has not all been removed. We hope to report our own investigations in this direction in a future paper.

What relation, if any, exists between these findings under pathological conditions and the thermostabile anticomplementary constituants of normal blood serum of Noguchi[17] and Manwaring[18] future investigations will have to show. A demonstration of their identity would of course constitute a very serious obstacle to the acceptance of v. Bergmann's view.

[17] Noguchi, H. The Thermostabile Anticomplementary Constituents of the Blood. *Journ. of Exper. Med.*, 1906, viii, 726.

[18] Manwaring, W. H. The Third Serum Component. *Journ. of Infect. Diseases*, 1906, iii, 647.

AN ANALYSIS OF FOUR HUNDRED CASES OF EPIDEMIC MENINGITIS TREATED WITH THE ANTI-MENINGITIS SERUM.*

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INTRODUCTION.

We have already reported concerning the effects of the employment of an antimeningitis serum, prepared in the horse by the inoculation of *Diplococcus intracellularis* and its products, upon the course and termination of a small number of cases of epidemic meningitis.[1] The results first reported were, on the whole, so satisfactory that we believed the employment of the serum on a wider scale not only justified but clearly called for; and we are now in position to present a second series of figures which are based upon an analysis of something more than 400 cases of epidemic meningitis in which the serum has been used.

The cases of meningitis upon which this analysis rests have arisen in different and widely separated parts of the United States and Canada, and in Great Britain. They have occurred sometimes as small epidemics, as in Castalia and Akron, Ohio, in Porterville, California, and possibly in other places in the United States, and in Belfast, Ireland, and Edinburgh, Scotland; and sometimes as sporadic outbreaks of considerable extent, as in Cleveland, Boston, Baltimore, Cincinnati, Philadelphia and New York. Moreover, it is now evident that so-called epidemic meningitis is widely prevalent throughout the United States, and it would appear to be question-

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[1] *Journal of Experimental Medicine*, 1908, x, 141. Independent publications on the use of the serum have been made by Robb, *British Medical Journal*, 1908, i, 382; by Dunn, *Boston Medical and Surgical Journal*, 1908, clviii, 370; and *Journal of the American Medical Association*, 1908, li, 15; by Chase and Hunt, *Archives of Internal Medicine*, 1908, i, 294; by Churchill, *Journal of the American Medical Association*, 1908, li, 21; and by Miller and Barber, *idem*, 1908, l, 1957.

able whether any parts are really free from the disease. In view of the fact that we have demanded that the bacteriological diagnosis be made in every case of meningitis for which we have supplied the serum, and which we have accepted for our analysis, and that in doubtful instances we have ourselves examined stained slides and sometimes cultures prepared from the spinal exudates, we can speak with positiveness upon this important subject. Whether the wide distribution of the epidemic type of meningitis in the United States is the outcome and residue of the epidemic that raged in New York and vicinity from 1905 to 1907, or whether the disease tends to exist and has long existed in a sporadic state in this country, from which the severe epidemic outbreaks have occasionally taken their origin, has not been satisfactorily determined.

The mortality of the disease reached during the height of epidemics in the United States and in Great Britain has been about the same, that is, 75 per cent. The mortality of the sporadic form of the disease so far as it has prevailed in the United States on a scale sufficiently large to allow of a conclusion upon this point, has not been considerably lower than that figure, and it has sometimes been higher. Dunn[2] gives a chart in which the mortality of the disease is shown for the cases treated in the Boston Children's Hospital from 1899 to 1907, before the antiserum was employed, and it ranges from 69 to 80 per cent. This chart also shows the striking fall in the curve which has been produced by the employment of the serum, for since the spring of 1907, when the use of the serum was first introduced, the mortality has fallen below 20 per cent. Meningitis has not been epidemic in Boston since 1897. The epidemic, properly speaking, of meningitis ceased in New York with the appearance of warm weather in the spring of 1907, and the cases of the epidemic type of the disease which have appeared since that time have arisen sporadically. We have procured from Dr. S. J. Baker, of the department of health, the case mortality statistics for this period. During 1906 when the epidemic was at its height 1032 cases with 812 deaths were reported in Greater New York; the mortality was 78.7 per cent. During 1907 when the epidemic was declining 828 cases with 642 deaths were reported; the mor-

[2] *Journal of the American Medical Association*, 1908, li, 16.

tality was 77.5 per cent. During the first six months of 1908 when the disease prevailed sporadically 253 cases with 182 deaths were reported; the mortality was 71.9 per cent. Dr. Baker informed us that the case mortality records of the department are less perfect than the gross mortality records; but as the average error of each period may be assumed to be the same, the figures may be accepted as generally correct. No striking diminution in the fatality of the disease has therefore taken place in Greater New York during 1908 as compared with the period corresponding to the height of the epidemic. The small epidemic which prevailed at Akron, Ohio, in 1907, consisted of 22 cases.[3] Among the first ten cases which occurred there were nine deaths: a mortality of 90 per cent. Then the use of the serum was begun, and it was employed in the next 12 cases, of which nine recovered: a mortality of 25 per cent. Of the three cases which were fatal one was moribund when first injected with the serum, and another suffered a fulminant attack. The epidemic at Porterville, California, began in December, 1907, and embraced 16 cases.[4] Among the first twelve cases there was one recovery: a mortality of 91.6 per cent. The serum was employed in the last four cases, of which three recovered: a mortality of 25 per cent. There was no difference observed in the manner of onset of the last four as compared with the earlier cases, although their course was milder and the termination more favorable. The epidemics in Ireland and Scotland have been of great severity, and the mortality, before our serum was employed, was above 70 per cent. During the first period of the use of the serum the mortality of cases observed outside and inside hospitals, but treated *without serum,* ranged from 80 to 90 per cent. The figures contained in Dr. Robb's report of 71 serum-treated cases give, after deducting the fulminant and moribund cases, 20 per cent., or including them, 26.7 per cent, mortality; Dr. Ker's figures, based on 27 cases, are higher, from 40 to 44.5 per cent., under the same conditions of calculation as the former.

The analysis which is to be presented is based upon histories of cases of epidemic meningitis in which the diagnosis has been estab-

[3] Chase and Hunt, *op. cit.*

[4] Miller and Barber, *op. cit.*

lished by bacteriological examinations as well as by the usual clinical tests. The histories have been supplied by physicians in hospital and in private practice, who have employed the serum. We are under many obligations to these physicians, who so generously gave their time to the study of the effects of the serum. It will not be possible for us to mention by name all who participated in the study. But our special thanks are due Dr. A. Gardner Robb, of Belfast, Ireland; Dr. C. B. Ker, of Edinburgh, Scotland; Dr. L. W. Ladd, of Lakeside Hospital, Cleveland; Drs. W. T. Longcope and Morris J. Lewis, of the Pennsylvania Hospital, and Dr. Franklin Royer, of the Municipal Hospital, Philadelphia; Drs. Charles H. Dunn, John Lovett Morse, J. L. Ames and Frederic Shattuck, Boston; Dr. Frank Fulton, of the Rhode Island Hospital, Providence; Drs. L. F. Barker, Frank J. Sladen and Harvey Cushing, of the Johns Hopkins Hospital, Baltimore; Drs. James D. Morgan and W. W. Wilkinson, of the Garfield Hospital, and Dr. S. S. Adams, Washington; Drs. Henry Koplik, Henry Heiman, Morris Manges and Alfred Meyer, of the Mt. Sinai Hospital, Dr. L. Emmett Holt, of the Babies' Hospital, Dr. C. H. Lewis, of St. Vincent's Hospital, Dr. G. M. Swift, of St. Mary's Hospital, Dr. George L. Peabody, of the New York Hospital, Drs. Walter James, John A. Thacher and W. T. Northrup, of the Presbyterian Hospital, New York; Dr. Charles W. Duval, of the Montreal General Hospital, Montreal; Drs. Frank S. Churchill and Maximilian Herzog, of Chicago; Dr. Alfred I. Cole, of the City Hospital, Cincinnati; Dr. Philip King Brown, of San Francisco, and Drs. Austin Miller and S. A. Barber, of Porterville; and the other physicians whose names are given in the table, attached to the isolated cases treated by them, and to the hospital internes who contributed such valuable service to the carrying out of the serum injections and the making of bacteriological and hæmatological examinations.

In making up the tabulations upon which the results are based, account has been taken of the ages and sex of the patients, the type of the disease, the period of the disease at which the serum was first injected, the days of the disease on which subsequent injections were made, the total amount of serum employed, the effects produced on the temperature and the subjective and objective symptoms of the

disease, upon the duration of the symptoms, the number of the diplococcus in the spinal exudate, and its viability in cultures, the general leucocytosis, and the manner of recovery—that is, whether by gradual improvement of the symptoms, or lysis, or by their abrupt termination, or crisis—and some other details of the disease.

We wish to state that Table I (see page 711 *et seq.*) contains a record of all the histories of cases of epidemic meningitis treated with the serum which were received by us up to the time of the completion of this report, and that in making up the various statistical tabulations no selection of cases has been made further than to exclude first, those cases which survived the first dose of the serum less than twenty-four hours. It may, we think, be accepted as probable that any marked benefit which the serum may be assumed to exert, could hardly be effectively manifested before the first twenty-four hour period following its administration had elapsed. It has chanced that of the histories here analyzed the eliminations include chiefly cases which were moribund at the time of their admission to hospital and the first serum injections, and in which the survival was often only a few hours. A second elimination, embracing a smaller number of cases, includes the rapidly fatal fulminant cases. As a rule it is possible to separate by means of the patients' histories and the clinical appearances, the fulminant cases which terminate fatally, from the moribund ones which have only a few hours to live. Indeed the propriety of eliminating the fulminant cases from the statistical calculations may possibly be a questionable procedure, since, rarely, a case with fulminant onset has recovered under the employment of the serum. However, in adhering to our rule not to take into account in calculating the percentage any case which survived a first serum injection less than twenty-four hours, the fulminant cases are practically eliminated. A third small elimination includes several cases of secondary and mixed infection of the meninges, of intercurrent infection (erysipelas, peritonitis) which were the immediate causes of death, and hopelessly chronic examples of the disease in children who were moribund after two to three months illness, but who survived a first serum injection longer than twenty-four hours. There were 21 cases eliminated as moribund, 12

cases as fulminant, and 10 as secondary and intercurrent fatal infections, or chronically moribund.

RESULTS ACCORDING TO THE AGES OF THE PATIENTS.

In Table I all the cases subjected to analysis are briefly described; they number 421. Of these, 43 cases were eliminated for the reasons already stated. Hence the total number of cases which will be subjected to analysis is 393, of which 295 or 75 per cent. recovered, and 98, or 25 per cent. died. Tabulated according to the ages of the patients the following results are obtained:

TABLE II.

	Total Number of Cases.	Recovered.	Died.	Mortality Percentage.
Under 1 year.	22	11	11	50
Between 1 and 2 years.	19	11	8	42.1
" 2 " 5 "	68	52	16	23.5
" 5 " 10 "	79	70	9	11.4
" 10 " 20 "	105	80	25	23.8
Over 20 years.	87	64	23	26.4
Age not given.	13	7	6	46.1

It will be desirable to consider these figures somewhat in detail. Perhaps the most striking figures are those relating to children under one year of age. Epidemic meningitis is commonly regarded as being uniformly fatal among infants under one year. Holt[5] states that "of twenty cases under one year (in his hospital wards) not one recovered"; and Koplik[6] states that of 27 cases below one year of age observed by him 23 either died, or were discharged unimproved. We have, therefore, made a special table of the cases of infants under one year of age, so that they may be more closely scrutinized.

The results shown in Table III are, indeed, not only instructive but of hopeful augury. Of the eleven cases which terminated in death, ten were already in the third week of the disease, or in a later stage, even, six or more presented well-marked symptoms of hydrocephalus, and one case only was in the first week of illness, when the serum injections were begun. Of the eleven

[5] Holt, Diseases of Infancy and Childhood, 1906, p. 763.

[6] Osler's Modern Medicine, 1907, ii, p. 575.

cases which recovered, one infant was four weeks old when first injected, all but three were in the first week of the disease, and none showed symptoms of hydrocephalus when the injec-

TABLE III.

Number.	Age in Months.	Sex.	Day of First Injection of Serum.	Total Quantity in c.c. of Serum Injected.	Result.	Reported by	Remarks.
1	2	?	120	60	Death 137th day.	Cushing.	Chronic hydrocephalus intraventricular injection.
2	5	M.	22	72	Death 29th day.	Mt. Sinai, N. Y.	Semi-chronic.
3	10	F.	15	35	Recovered.	" "	Rapid recovery.
4	3½	M.	20 (?)	102	Death in 5th week.	" "	History imperfect; possibly later injection.
5[7]	5	M.	49	30	Death.	Garfield, Hosp.	Hydrocephalus and mixed infection on admission.
6[7]	11	M.	21	135	"	New York Hosp.	Hydrocephalus; erysipelas.
7	11	F.	49 (?)	47	Death 117th day.	Babies' Hosp.	Chronic case.
8	6	M.	23	60	Recovered.	" "	Prompt recovery.
9	6	M.	(?) late	29	Death.	Lakeside Hosp.	Hydrocephalus on admission.
10	7	M.	6	60	Recovered.	" "	Slow recovery.
11	3	M.	14 (?)	90 (?)	Death.	" "	Complicated by general œdema on admission.
12	11	M.	2	30	Recovered.	Cincinnati Hosp.	Prompt recovery.
13	1	F.	2	16	"	" "	" "
14	3½	F.	7	75	"	Dunn, Boston.	" "
15	11	F.	21	60	"	" "	" "
16	5	M.	4	45	"	" "	" "
17	8	M.	23	45	Death.	Robb, Belfast.	Semi-chronic.
18	3	M.	5	150	Recovered.	" "	Slow recovery.
19	9	F.	3	90	"	" "	Prompt recovery.
20	4	F.	42	184	Death.	Morse, Boston.	Hydrocephalus. No fluid by lumbar puncture.
21	6	M.	60	60	"	Newark Hosp.	Chronic case.
22	7	M.	7	30	Recovered.	Adams, Washington.	Prompt recovery.
23[7]	5	M.	7	108	Death.	Bellevue, N. Y.	Secondary streptococcus infection.
24	4	F.	21	134	"	Jennings, N. Y.	Hydrocephalus.
25	10	M.	5	75	"	Ker, Edinburgh.	Severe case.

tions were begun. Hence the outlook for favorable effects from the serum injections in infants under one year of age may be considered hopeful, provided the injections are begun at an early period of the infection and before anatomical changes leading to

[7] Cases eliminated from the tabulation.

hydrocephalus, and the consequent locking up of infected exudate in the cerebral ventricles, are established.[8]

The mortality among somewhat older children—those ranging from one to two years of age—has been in the past as high or almost as high as among the younger infants. Of the nineteen children in our series between the ages of one and two years, eleven recovered and eight died. An analysis of these shows that only one case injected in the first week of illness died, while six cases injected in the first week, and five cases injected from the second to the fourth week, recovered. It is probable therefore that the same conditions determine for this series as for the preceding series what the outcome shall be, and the chief obstacle to successful treatment by the serum injections is to be found in the greater tendency exhibited by young children affected with epidemic meningitis to develop early basilar lesions leading to chronic hydrocephalus.

The rise in recoveries and fall in deaths in the next several series of cases, until the twentieth year is reached, call for no special comment, but show conclusively that the mortality from epidemic meningitis can be greatly reduced by the proper injection of an antiserum. We are, indeed, encouraged by the figures presented even beyond what they actually exhibit, since we believe that the cases have not been treated uniformly as well as they might have been. As will be shown presently when we come to speak of the influence of the period of injection upon the mode of termination of the disease, one of the important factors determining good results is early injection, and yet in many cases, where the outcome was a good one, the injections were made late in its course. Aside from this fact, however, are the additional facts of the skill with which the serum is injected, and its proper dosage and spacing, regarding which there have been unavoidable discrepancies. In two instances at least a fatal termination was insured and recovery de-

[8] Possibly even this condition of retained infected exudate in the cerebral ventricles may not be hopeless now that Cushing and Sladen have shown the feasibility of direct drainage of the ventricles and serum injection. See *Jour. of Exp. Med.*, 1908, x, 548. Since the completion of this report we have received from Dr. Robb the histories of three cases in children under one year of age and one case in a child fifteen months old, in all which the serum was injected in the first week of illness and recovery took place.

feated, apparently, through the induction from without of secondary pyogenic infection of the meninges. In one case a convalescent succumbed to a *Staphylococcus aureus* infection arising from an infected needle track, and in another a patient who had shown rapid improvement succumbed to a streptococcus infection also of external origin.

The possibility of this danger of secondary infection of the meninges from without should always be kept in mind by those entrusted with the injection of the serum, and no pains should be spared in disinfecting the field of operation and all objects coming into contact with the serum. Every precaution should be taken from the beginning, since prediction seems, at present, impossible, whether one or many injections of the serum may be required. Cases of marked severity have improved so rapidly after one injection as not to demand another, and cases apparently mild have called for successive injections before responding to the treatment. The general statement may be hazarded that the milder the case and the earlier the injection of serum is made, the more certain and more rapid the improvement; but to this statement there have already occurred not a few exceptions in which severe and semi-chronic cases have responded more quickly and surely than earlier mild ones. The individual factors of patients and of micro-organisms play a part here as in all other infective diseases with which we are familiar.

In our first publication we pointed out the dangers and difficulties that are connected with the intradural mode of administration of the serum which we then regarded as essential; and, as may now be added, the wider our experience with the serum has become, the more have we been convinced that the successful substitution of subcutaneous or intravenous for intraspinal injection cannot be accomplished.

We wish to draw especial attention to the number of deaths among the cases embraced in the series over twenty years of age, since we believe that the somewhat higher mortality among them can be explained, probably, by the fact that a larger number than in other series was treated by widely scattered physicians who had had little or no previous experience with the serum. If this is not

the reason, and adults of twenty years and over are less subject to the beneficial action of the serum than younger persons, the fact will of course come out finally; but with one exception (Cincinnati City Hospital series) wherever a considerable number of cases of these ages has been treated by one person the proportion of recoveries to deaths has been high. In justice to Dr. Cole, of Cincinnati, whose general results obtained with the serum have been excellent, it should be stated that many of the failures occurred in the early period of his employment of the serum and among negro patients, the circumstances surrounding which made the most favorable administration of the serum difficult. In all Dr. Cole treated with the serum eleven cases over twenty years of age. Of these two were moribund and died soon after the first injection. The other six fatal cases were injected between the third and twentieth days of illness. The three cases which recovered were injected in the first week of illness. These figures are to be contrasted with those obtained at the Johns Hopkins Hospital, where of seven patients over twenty years of age five recovered and two died, and especially with those given by Dr. Robb, of Belfast, who treated twenty-one cases of these ages, eighteen recovering; and of the three fatal cases two died within twenty-four hours of the first injection of serum. Dr. Robb's cases are especially significant, since they were treated during the prevalence of an epidemic of meningitis.

RESULTS ACCORDING TO THE PERIOD OF INJECTION.

We have also analyzed the histories according to the earliest periods of the disease at which the injection of the serum was begun. Not all the histories are perfectly definite on this point and hence we have used in the analysis only those that are definite. In not a few cases the onset of the disease was insidious and the prodromata appear to have been indefinite, and more or less overlooked. At other times, and this seems to have been the more frequent experience, the onset was abrupt, so that no special doubt surrounded the beginning of the disease. Under the circumstances, therefore, the danger is that the period elapsing between the onset of the symptoms of the disease, their recognition, and the first

serum injection, will be calculated too short rather than too long. It is very rare, except in the fulminant cases, that one can assure himself that he is dealing with the disease on the first day of its existence.

The histories of 361 cases were sufficiently explicit to enable us to approximate the period in which the first serum injection was made. We have arbitrarily chosen the three periods which follow, in which to group the cases:

Period of Injection of Serum.	Number of Cases.	Number Recovered.	Number Died.	Per Cent. Mortality.
1st to 3d day.	123	107	16	16.5
4th "7th "	126	96	30	23.8
Later than 7th day.	112	73	39	35

In spite of the uncertainties surrounding the period of onset of the symptoms, which affect the accuracy of the calculation of the period, the beneficial influence of early injection is rendered sufficiently obvious by the table. The period embraced in the last group is, of course, highly irregular, since not a few cases came under treatment when they were in a semi-chronic or chronic state after many weeks of illness. On the whole, therefore, the outlook even for the latter class of patients is not wholly discouraging; and, indeed, we are of the opinion that so long as the diplococcus is still present in the meningeal exudate, and the mechanical damage to the anatomic structure is not irreparable, the employment of the serum holds out hope of considerable benefit. In one respect cases coming under any treatment at the end of a week or even a longer interval since the appearance of the symptoms of meningitis, present the advantages which accrue from the spontaneous elimination by death of the severer and rapidly-fatal examples of the disease, and the circumstance that some of them will already be progressing towards recovery. The offset to these advantages is to be found in the larger number of cases of the common and severer types, which, in the past, having survived the early acute stage of the disease, developed semi-chronic and chronic lesions to which they succumb. Hence in any considerable number of cases of the class under consideration the fatalities have up to the present tended

greatly to exceed the recoveries. We should, therefore, expect less benefit to be manifested by the serum injections which are carried out during the later, as compared with the earlier periods of the infection. In the next table (IV) the results of the periods of injection in the different age groups are shown.

TABLE IV.

Age.	1st to 3d Day.				4th to 7th Day.				Later Than 7th Day.			
	No. Cases.	Rec.	Died.	Per Cent of Deaths.	No Cases.	Rec.	Died.	Per Cent of Deaths.	No. Cases.	Rec.	Died.	Per Cent of Deaths.
Under 1 yr.	3	3		0	6	5	1	16.7	12	4	8	66.7
1 to 2 yrs.	3	3		0	4	3	1	25	12	6.	6	50
2 to 5 yrs.	17	15	2	11.7	34	24	10	29.5	17	13	4	23.5
5 to 10 yrs.	38	35	3	8.6	25	21	4	16	12	10	2	17
10 to 20 yrs.	42	37	5	12	32	26	6	23	27	17	10	37
Over 20 yrs.	20	14	6	30	25	17	8	32	32	23.	9	28.1
Total	123	107	16	16.5	126	96	30	23.8	112	73	39	35

MANNER OF TERMINATION OF THE SYMPTOMS.

We described in our first publication[9] on the antimeningitis serum the different manner in which the symptoms terminated in a number of the serum-treated cases, and we drew attention to the not uncommon abrupt manner of termination to which we applied the term crisis. We have examined the larger collection of histories now in our possession from the point of view of the manner of the recovery; that is, whether by gradual subsidence of the symptoms, or lysis, or by the abrupt cessation, or crisis. In considering the figures which we will present as bearing on this point, account should be taken of the fact that in rare instances only have the histories contained the specific statement that the symptoms terminated in one or the other of these ways. In almost all instances we have had ourselves to make the decision, which we have done by studying carefully the facts given in the histories. Hence we do not believe that our decisions have been uniformly correct, and the figures are presented, therefore, merely as an approximation of what may be found later to be the true figures. There can be little doubt that these figures will have to be modified later, in accord-

[9] *Op. cit.*, p. 197.

ance with the wider observation and more exact and uniform judgment which will come to be exercised. We believe, however, that by the employment of the serum a new and very desirable mode of recovery, to secure which in the future every effort should be made, has been effected.

In order that recovery by crisis should be intentionally promoted it will be necessary, as a preliminary step, to study closely the conditions under which it has come about spontaneously in the serum-treated cases, so that they may be designedly imitated. We do not ourselves feel able to undertake this study, which should, we think, be made by the clinicians themselves, but we think it desirable, imperfect as the present figures may be, to classify them in a way that may serve to bring out their suggestive values. We have, therefore, brought them together in the form of a table (V), and arranged them according to the ages of the patients and the three periods of the first injection of the serum.

TABLE V.

	First to Third Day.	Fourth to Seventh Day.	Later than Seventh Day.	Total.
Under 1 yr.				
By lysis.	3	5	1	9
By crisis.	0	0	1	1
1 to 2 yrs.				
By lysis.	3	3	4	10
By crisis.	0	0	1	1
2 to 5 yrs.				
By lysis.	10	19	10	39
By crisis.	5	5	3	13
5 to 10 yrs.				
By lysis.	24	15	7	46
By crisis.	11	6	3	20
10 to 20 yrs.				
By lysis.	24	19	13	56
By crisis.	13	7	4	24
Over 20 yrs.				
By lysis.	10	11	19	40
By crisis.	4	6	4	14

The above table (V) shows that of 273 cases of epidemic meningitis treated with the serum which recovered, the termination of the symptoms in approximately 200 was a gradual one and in 73 more or less sudden in nature. And, hence, allowing for unavoid-

able errors of judgment in interpreting the histories, we think that it is probable that this abrupt or critical cessation of the symptoms may take place in about 25 per cent. of the patients who recover under the influence of the serum treatment. Subjecting the figures in the table to a still closer scrutiny, it is found that the number of instances of lysis and crisis occurring in cases treated within the first three days of illness is in the proportion of 74 to 33, on the fourth to seventh day, of 72 to 24, and later than the seventh day, of 54 to 16. In other words the ratio according to these periods is 2.3, 3 and 3.4 to one respectively. It will not be profitable to carry this analysis further, since the table exhibits clearly other relationships and the whole number of cases is not large enough to warrant the making of any far-reaching deductions.

FREQUENCY OF RELAPSES.

It was evident to us, from the study of the first series of cases treated with the serum, that a certain number of cases, apparently recovering, would suffer recrudescences of the disease. At that time no further statement could be ventured than that the resumption of the serum injections promised to control this condition of relapse as they had the original attack.[10] The present larger number of histories has afforded some additional data bearing on the subject of the relapses, but they are, as affecting this subject, of very unequal value. We have been able to collect from the histories 19 examples of undoubted relapse, of which 14 terminated in recovery and 5 in death. We are, therefore, encouraged to believe that the early recognition of the relapses, and a prompt and vigorous resumption of the injections, will often arrest their progress and bring about a favorable termination of the disease.

DURATION OF ACTIVE SYMPTOMS.

Closely connected with the question of the manner of termination of the symptoms is the question of the duration of the active symptoms of the disease in serum-treated as compared with non-serum-treated cases. In view of the relatively large number of the former cases in which the symptoms cease abruptly, the fact is open to

[10] *Op. cit.*, p. 199.

prediction that the average duration in days, of the active symptoms of any considerable number of the cases, will be smaller than of a similar number of cases which had not the benefit of the serum. We have analyzed 228 histories, in which the data covering this point are pretty complete, with reference to the duration of the fever and the usual subjective and special objective symptoms among patients who recovered, and found the period to be about eleven days.

This period, it should be stated, embraces not the entire time during which the patients were under observation in hospitals and private practice before their discharge, but it covers the interval during which they presented *active* symptoms of illness. Hence it has happened that the interval has not infrequently been estimated as only a few days in length, although the recovery of lost strength and the ability to resume the ordinary vocations took a much greater number of days.

INFLUENCE ON DIPLOCOCCI.

In our first publication on the serum treatment of epidemic meningitis we drew attention to a fact, which impressed us as remarkable and significant, namely, that very soon after the serum injections were begun the diplococci tended to be greatly reduced in numbers, to disappear from the fluid part of the exudate, to become wholly intracellular (unless they were already entirely absent), to present certain changes in appearance, as swelling and fragmentation, and to stain diffusely and indistinctly, and coincidentally to lose viability in cultures. The later and far wider experience has tended to confirm the views we first expressed, based on the effects observed; and while exceptions occur in which the diplococci disappear or become engulfed and change in morphology or lose viability more slowly, yet the general fact of the profound and rapid injury exerted upon the diplococci by the serum seems securely established. There seems little doubt that part of the beneficial effect of the serum injections must arise from the restriction of multiplication and from the greater phagocytosis of the diplococci.

We have indicated in our main table (I), under the heading "Influence on Diplococci," what the fate of the diplococci in the

spinal exudate was in cases in which the serum was injected, and in which adequate observations were made on the number, appearance, location, and viability of the diplococci. By the phrase "rapid" or "prompt disappearance and loss of viability" of the diplococci, we mean that after the first, second or third serum injection they were greatly reduced in numbers and lost power to grow on favorable culture media, and by the term "slow loss of viability" or "gradual" or "slow disappearance," we mean that the diplococci showed themselves more resistant to the serum. Perhaps the strongest impression of the rapidly injurious action of the serum upon the diplococci will be brought out by a simple statement of the number of instances in which, recovery having taken place, the diplococci were affected in this "rapid," or "prompt," and in this "slow" manner. The records in the histories of 110 cases are sufficiently complete to admit of a decision upon the fate of the diplicocci; and we have therefore ascertained that in 100 cases the diplococci disappeared and lost viability quickly, and in 10 cases slowly.

Perhaps a word should be added on the question of the value as evidence of the observed loss of power of the diplococci to grow in the cultures, on their capacity to multiply in a restricted manner in the meningeal exudate. We believe that there is no absolute agreement between the facts regarding cultivation outside the nervous system and capacity for growth in the meninges. Instances have been observed (Presbyt. Hosp. Case 2, etc.) in which relapse of symptoms was ushered in, or attended by, increase in the number and resumption of normal appearance of the diplococci, which yet did not grow in cultures, although the medium was otherwise favorable to the diplococcus. It is of course in no degree surprising, but quite what should, in view of the anatomical conditions present, be expected, that after complete disappearance of the diplococci from the spinal exudate they should sometimes reappear. The resolution of the fibrino-purulent exudate must often proceed slowly, and until it is complete the diplococci must often find favorable niches in which to survive and even to multiply. From such active foci the reinfections, with which the relapses are associated, probably take place. In the fatal cases the diplococci are often more resistant, and fail wholly to be influenced by the serum injections, or are

little influenced by them. The viability under these circumstances is likely also to be retained.

The opportunities for such cryptic survival of the diplococci probably explain the exceptional instances in which the symptoms and the character of the spinal exudate leave no doubt of the existence of acute (epidemic?) meningitis, but in which there is failure to demonstrate the diplococci at the first lumbar puncture, or even at all in cases which recover rapidly.

INFLUENCE ON SPINAL EXUDATE AND LEUCOCYTOSIS.

Attention was previously directed by us to the rapidity with which the exudate in the meninges loses turbidity under the influence of the serum injections.[11] This fact has been noted again and again in the subsequent cases treated with the serum. Indeed, it would now appear as if the fear we expressed that the cases with strictly purulent exudates might be less amenable to the action of the serum was premature. A fair number of cases (see Table I) in which the notes state the spinal exudate to have been purulent, have recovered, and the rapid clearing of the exudate was observed even in them. Whether there is complete anatomical restitution of the meninges in these cases can only be determined by post-mortem examinations; but that complete functional restoration can take place may be regarded as established.

Closely connected with the rapidity with which the cerebro-spinal exudate loses pus cells and returns to a limpid condition is the state of the general leucocytes of the blood. If the inflammatory emigration is arrested by the serum injections, then the number of circulating leucocytes should tend rapidly to return to the normal. The facts at hand, based upon many counts of the circulating leucocytes, only a few representative ones of which can be reproduced here, made before the injections of the serum were begun, and afterwards at regular intervals, show, as was to be expected, in favorable cases going on to recovery, a fall, often very rapid and even critical, in the number of leucocytes in the general blood stream with which the disappearance of the diplococci and the clearing of the spinal exudate are correlated.

[11] *Op. cit.*, p. 200.

Case.	Age.	Before Injection.	After First Injection.	After Second Injection.	After Third Injection.	Result.
Johns Hopkins Hospital No. 1.	6 yr.	21,000 (Dec. 24). 37,600 (Dec. 28).	Serum, Dec. 30. 15,600 (Dec. 31).	Serum, Dec. 31. 20,400 (Jan. 2).	Serum, Jan. 2. 8,000 (Jan. 23).	Recovered.
Idem No. 14.	11 yr.	40,600 (Apr. 2).	Serum, Apr. 2. 31,200 (Apr. 3).	Serum, Apr. 3. 13,400 (Apr. 5).		Recovered by crisis.
Idem No. 19.	15 yr.	44,000 (May 20).	Serum, May 20. 26,400 (May 21).	Serum, May 21. 6,400 (May 24).		Recovered by crisis.
Mt. Sinai No. 11.	14 yr.	32,600 (Mar. 10).	Serum, Mar. 11. 28,000 (Mar. 12). 25,000 (Mar. 13).	Serum, Mar. 13. 16,000 (Mar. 14). 15,000 (Mar. 16).		Recovered.
St. Mary's Hospital No. 2.	12 yr.	47,500 (Mar. 21).	Serum, Mar. 21. No count.	Serum, Mar. 22. 12,500 (Mar. 23).		Recovered by crisis.
Pennsylvania Hospital No. 9.	23 yr.	23,350 (May 14). 20,650 (May 16).	Serum, May 16. 10,550 (May 17). 12,050 (May 19). 10,600 (May 20). 12,500 (May 21).	Serum, May 21. 24,950 (May 22).	Serum, May 22. 12,450 (May 23). 10,900 (May 24). 9,600 (May 25). 13,400 (May 26). 10,750 (May 27). 10,850 (June 9).	Recovered

It has been stated already that during the relapses, and possibly as ushering them in, the spinal exudate becomes more turbid, with which condition there is correlated an increase in the systemic leucocytosis, as is shown by the following example (p. 708).

The reverse of the phenomena here mentioned is encountered in those cases not responding to the serum, or responding imperfectly, in which death is the result. Although the data bearing on this topic at our command are less numerous and perfect than the other, yet the general statement can be made that the diplococci, the spinal exudate, and the circulating leucocytes are less influenced in the

Case.	Age.	Before Injection.	After First Injection.	After Second Injection.	After Third Injection.	After Later Injections.	Result.
Presbyterian Hospital, N. Y. No. 2.	4 yrs.	No count.	Serum Jan. 25. 39,700 (Jan. 26) 34,800 (Jan. 27)	Serum Jan. 27. 22,500 (Jan. 29)	Serum Jan. 29. 16,400 Jan. 30.	Serum Jan. 30. 22,500 (Feb. 1) Serum Feb. 2. 17,300 (Feb. 3) 25,200[12] (Feb. 4) Serum Feb. 4. 28,200[12] (Feb. 6) Serum Feb. 6. 24,200 (Feb. 7) Serum Feb. 7. 19,100 (Feb. 8) Serum Feb. 8. 14,000 (Feb. 9) Serum Feb. 10. 13,500 (Feb. 11) 9,900 (Feb. 15)	Relapse with recovery.

resistant cases, and that progressive increase in turbidity of the exudate, and rise in leucocytosis, and greater persistance of the diplococci, with retention of viability, are unfavorable indications. The relation of a persistently high or increasing systemic leucocytosis, and an unfavorable termination of the disease is shown by the next examples (p. 709).

FREQUENCY OF PERMANENT SEQUELÆ.

The indications which were given by the first series of serum-treated cases were to the effect that the recoveries, as the rule to which the exceptions would not be numerous, would be complete. The facts brought out by the far larger series of cases on which this article is based confirm the earlier view which we expressed. The

[12] Appearance of relapse.

Case.	Age.	Before Injection.	After 1st Injection.	After 2d Injection.	After 3d Injection.	After Later Injections.	Result.
New York Hospital, No. 1.	18 mo.	30,000 (Dec. 2).	Serum, Dec. 2. 20,400 (Dec. 3).	Serum, Dec. 3. 22,000 (Dec. 4).	Serum, Dec. 4. 21,800 (Dec. 6).		Died.
Cincinnati Hospital, No. 26.	17 yr.	25,000 (Mar. 31).	Serum, Mar. 31. 21,000 (Apr. 1). 16,200 (Apr. 2).	Serum, Apr. 2. 16,460 (Apr. 4).	Serum, Apr. 4. 10,730 (Apr. 6). 26,000 (Apr. 7).	Serum, Apr. 7. 28,666 (Apr. 8). 26,200 (Apr. 9). 25,400 (Apr. 10). 31,460 (Apr. 11).	Died.
Adams, Washington, No. 1.	5 yr.	No count.	Serum, Feb. 11.	28,200 (Feb. 13). Serum, Feb. 13.	Serum, Feb. 14. 26,400 (Feb. 15).	29,800 (Feb. 16). Serum, Feb. 16. 42,400 (Feb. 18). 59,200 (Feb. 20). Serum, Feb. 20. Serum, Feb. 22. Serum, Feb. 24. 57,400 (Feb. 26).	Died.

complications of a serious character, arising during and as a result of the meningeal inflammation, which remained permanently after the infection and inflammation subsided, have been few. There are noted in the histories deafness seven times, blindness and deafness once (in an infant in whom they were already present on the twenty-second day, when first injected), mental impairment once, and choroiditis once. The condition of deafness was, almost invariably, noted early in the disease, and several times before the serum injections were begun.

CONCLUSION.

It is our belief that the analyses of histories of cases of epidemic meningitis which have been presented in this article furnish convincing proof that the antimeningitis serum when used by the sub-

dural method of injection, in suitable doses and at proper intervals. is capable of reducing the period of illness; of preventing, in large measure, the chronic lesions and types of the infection; of bringing about complete restoration to health, in all but a very small number of the recovered, thus lessening the serious, deforming, and permanent consequences of meningitis; and of greatly diminishing the fatalities due to the disease.

TABLE I.

R. L. = recovery by lysis.
R. C. = recovery by crisis.
R. R. = recovery by relapse.

D. = died.
D. F. = died in fulminant attack.
D. M. = moribund at time of injection.

D. R. = died in relapse.
D* = eliminated from tabulation for special reason.

Reported by	Case No.	Name	Sex	Age	Days of disease when serum injected	Total amount serum injected	Influence on temperature	Influence on special sympt.	Influence on Diplococci	Period of fever and sympt.	Type of disease and remarks	Result
Johns Hopkins Hospital, Baltimore	1	H. N.	M.	6 yr.	47, 48, 50	65	Gradual reduction	Prompt improvement	Rapid disappearance. No growth after first injection.	10 d.	Ordinarily severe, and protracted. Symptoms subsided quickly after serum injection.	R. L.
Idem	2	I. H.	M.	15 yr.	4, 6, 7, 13, 17	134	Gradual reduction	Gradual improvement	Prompt disappearance and loss of viability.	17 d.	Severe. Fluctuating improvement.	R. L.
Idem	3	G. F.	M.	29 mo.	13, 14	45	Prompt reduction	Prompt improvement	None.	.....	Ordinarily severe. Respiratory failure 12 hours after serum injection. Artificial respiration. Death 36 hours later.	D.
Idem	4	A. R.	M.	13 mo.	21, 22, 23, 31	105	Gradual reduction	Gradual improvement	Rapid reduction in number and loss of viability	10 d.	Insidious onset. Protracted course; rapid improvement after injection of serum.	R. L.
Idem	5	J. G. P.	M.	23 yr.	5, 6	30	Gradual reduction	Prompt improvement	Diplococci difficult to demonstrate.	12 d.	Insidious onset. Ordinarily severe.	R. L.
Idem	6	C. S.	M.	5 yr.	5, 6, 7	60	Gradual reduction	Rapid improvement	Rapid disappearance and loss of viability	6 d.	Ordinarily severe.	R. L.
Idem	7	G. H.	M.	26 yr.	2, 3 (two) 4 (two)	120	None	None	Reduction in number and loss of viability	.....	Very severe. Onset with convulsions. Died in convulsion 4th day.	D.
Idem	8	I. S. H.	M.	5 yr.	3, 4, 5, 7, 8, 10	138	Fall by crisis after 6th injection	Critical improvement after 6th injection	Great reduction after 3d injection.	7 d.	Ordinarily severe.	R. C.
Idem	9	M. G.	M.	7 yr.	5	30	Rapid reduction	Rapid improvement	Second puncture not made.	2 d.	Sudden and severe onset.	R. C.

TABLE I.—*Continued.*

Reported by	Case No.	Name	Sex	Age	Days of disease when serum injected	Total amount serum injected	Influence on temperature	Influence on special sympt.	Influence on Diplococci	Period of fever and sympt.	Type of disease and remarks	Result
Johns Hopkins Hospital,	10	J. D.	M.	7 yr.	14, 16	45	Rapid reduction	Rapid improvement	Rapid loss of viability	2 d.	Ordinarily severe.	R. C.
Idem	11	H. L. B.	M.	7 yr.	2, 3	60	Rapid reduction	Rapid improvement	Rapid loss of viability	4 d.	Severe onset.	R. C.
Idem	12	N. S.	F.	25 yr.	4, 5 (two)	90	None	None	Rapid loss of viability	.....	Insidious onset. Very severe. Death 5 days after severe symptoms appeared.	D.
Idem	13	B. N.	F.	9 yr.	4, 5, 6, 7	105	Gradual reduction	Gradual improvement	Rapid loss of viability	10 d.	Severe. Complicated with iridocyclitis.	R. L.
Idem	14	F. N.	M.	11 yr.	2, 3	60	Prompt reduction	Rapid improvement	Rapid loss of viability	5 d.	Ordinarily severe.	R. C.
Idem	15	P. J.	M.	27 yr.	7, 8, 9, 19	105	Gradual reduction	Gradual improvement	Rapid disappearance	14 d.	Insidious onset. Ordinarily severe.	R. L.
Idem	16	L. A.	F.	25 mo.	6, 7	40	Rapid reduction	Rapid improvement	Difficult to demonstrate	4 d.	Ordinarily severe.	R. L.
Idem	17	P. H.	M.	30 yr.	3 (two)	45	Rapid reduction	Rapid improvement	Rapid disappearance	3 d.	Ordinarily severe.	R. C.
Idem	18	T. W. B.	M.	6 yr.	2, 3	45	Prompt reduction	Prompt improvement	Rapid disappearance	5 d.	Severe onset.	R. L.
Idem	19	G. G.	M.	15 yr.	2, 3	45	Prompt reduction	Prompt improvement	Difficult to demonstrate	6 d.	Severe onset.	R. C.
Idem	20	?	F.	36 yr.	60 (?), 62, 64	45	Prompt reduction	Prompt improvement	Rapid disappearance	6 d.	Severe. Chronic state	R. L.
Idem	21	?	?	2 mo.	120 (?), 126, 129, 132	60	.....	Temporary improvement	Reduction after 3d injection	.....	Chronic case. Hydrocephalus. Diplococci absent from spinal fluid. Intraventricular injection of serum. Death on 137th day. (Reported by Cushing and Sladen.)	D.
Idem	22	H. D.	M.	28 yr.	6, 7	40	Rapid reduction	Rapid improvement	.....	4 d.	Ordinarily severe.	R. C.
Municipal Hospital, Philadelphia	1	B. F.	M.	8 yr.	7, 8, 12, 14, 31, 33, 35, 37	180	None	Temporary improvement	Rapid disappearance	.....	Ordinarily severe. Death on 53d day.	D.
Idem	2	C. F.	F.	18 yr.	4, 5	60	None	None	None	.....	Severe. Death on 6th day	D.

Idem	3	S. D.	F.	7 yr.	5, 6, 7, 8, 10	140	Rapid reduction	Gradual improvement	Rapid loss of viability	6 d.	Ordinarily severe.	R. L.
Idem	4	S. S.	M.	3 yr.	4 (two), 6, 8, 18, 20, 22, 24	200	None	None	None	.....	Severe. Death on 25th day	D.
Idem	5	P. D.	M.	10 yr.	2, 4, 6	90	Gradual reduction	Gradual improvement	Rapid loss of viability	2 w.	Ordinarily severe. Deafness.	R. L.
Idem	6	A. M.	F.	8 yr.	6	30	Rapid reduction	Rapid improvement	.....	2 d.	Ordinarily severe.	R. C.
Idem	7	H. B.	F.	4 yr.	9, 11, 13	90	Prompt reduction	Rapid improvement	Rapid loss of viability	7 d.	Ordinarily severe.	R. L.
Idem	8	J. C.	M.	22 yr.	13 (?), 14, 15, 16	95	Gradual reduction	Gradual improvement	.....	10 d.	Mild. History inadequate.	R. L.
Idem	9	R. R.	M.	13 yr.	3, 4, 5, 8, 11	150	Gradual reduction	Prompt improvement	Rapid disappearance	10 d.	Ordinarily severe.	R. L.
Idem	10	H. B.	M.	10 yr.	8, 9, 11	90	None	None	None	.....	Ordinarily severe. Death on 12th day.	D.
Idem	11	G. C. B.	F.	10 yr.	5, 6, 8, 10, 18	150	Rapid reduction	Gradual improvement	Rapid disappearance	23 d.	Ordinarily severe. Deafness.	R. L.
Idem	12	J. B.	M.	13 yr.	3, 4, 5, 7	120	Rapid reduction	Rapid improvement	Rapid disappearance	5 d.	Ordinarily severe.	R. C.
Idem	13	H. S.	F.	9 yr.	3, 4, 5, 8, 31, 32, 34	210	Rapid reduction	Gradual improvement	Reduction after 1st injection	.....	Severe with relapse. First attack lasted 7 days. Relapse occurred after interval of 3 weeks and lasted 5 days.	R. R.
Idem	14	E. F.	M.	8 yr.	5, 6, 7, 8, 10	150	None	None	Rapid disappearance	.....	Ordinarily severe. On 10th day an abscess developed in puncture wound and followed needle track into spinal canal. Death from S. Aureus infection.	D.*
Idem	15	M. W.	M.	1 yr.	10, 12	30	None	None	.....	.....	Severe onset. The 1st injection only entered canal. Death in 5th week.	D.*
Idem	16	T. C.	M.	4 yr.	5, 6, 7, 8, 9, 10	165	Gradual reduction	Gradual improvement	Slow disappearance	2 w.	Ordinarily severe.	R. L.
Idem	17	A. W.	M.	44 yr.	4 (two), 5, 6	120	None	None	Prompt reduction	.....	Very severe. One injection only certainly entered canal. Died 6th day.	D.*
Idem	18	E. M.	M.	adult	4 injections on successive days	90	Gradual reduction	Gradual improvement	Rapid disappearance	6 d.	Ordinarily severe.	R. L.
Idem	19	J. V.	M.	adult	1	30	.....	.....	.....	.....	Small amount of pus obtained on puncture. Death occurred within one hour of injection.	D. M.

TABLE I.—*Continued.*

Reported by	Case No.	Name	Sex	Age	Days of disease when serum injected	Total amount serum injected	Influence on temperature	Influence on special sympt.	Influence on Diplococci	Period of fever and sympt.	Type of disease and remarks	Result
Municipal Hospital, Philadelphia	20	F. D.	M.	child	3 (two)	60	.....	.....	.....	.....	Severe. Death occurred 10 hours after 2d injection.	D. M.
Idem	21	M. D.	F.	child	3, 4, 5, 6, 7, 8, 9	185	Gradual reduction	Gradual improvement	Rapid disappearance	10 d.	Severe. Sister of previous child.	R. L.
Mt. Sinai Hospital, N. Y.	1	M. L.	M.	5 mo.	22, 23, 25, 26	72	.....	None	Rapid loss of viability	.....	Severe. Death on 29th day.	D.
Idem	2	K. S.	F.	10 mo.	15	35	Rapid reduction	Rapid improvement	.....	6 d.	Severe.	R. C.
Idem	3	A. K.	M.	2 yr.	7	23	Rapid reduction	Rapid improvement	.....	7 d.	Ordinarily severe.	R. C.
Idem	4	S. M.	F.	5.5 yr.	21, 31	59	Gradual reduction	Gradual improvement	Slow disappearance	4 w.	Severe. Protracted course.	R. L.
Idem	5	L. S.	F.	24 yr.	19, 21, 23, 25, 28, 30	140	Gradual reduction	Prompt improvement	Prompt disappearance	12 d.	Severe.	R. L.
Idem	6	M. S.	M.	4 yr.	5	30	Prompt reduction	Prompt improvement	.....	10 d.	Ordinarily severe.	R. L.
Idem	7	H. B.	M.	15 mo.	23	30	Gradual reduction	Slow improvement	.....	3 w.	Severe.	R. L.
Idem	8	H. L.	M.	3.5 mo.	20, 22, 26, 29, 32, 36	102	None	None	None	.....	Ordinarily severe. Death in 5th week.	D.
Idem	9	H. I.	M.	3 yr.	6, 13, 18, 27	75	Gradual reduction	Slow improvement	Rapid loss of viability	2 w.	Ordinarily severe.	R. L.
Idem	10	M. G.	M.	15 mo.	8	30	Rapid reduction	Rapid improvement	.....	5 d.	Severe.	R. C.
Idem	11	F. F.	M.	2.5 yr.	6, 7	88	Gradual reduction	Slow improvement	Rapid loss of viability	16 d.	Severe. 1st puncture yielded thick, greenish pus; 2d thin straw-colored fluid	R. L.
Idem	12	E. W.	F.	14 yr.	5, 7	55	Prompt reduction	Prompt improvement	Rapid loss of viability	6 d.	Ordinarily severe.	R. L.
Idem	13	E. G.	F.	4.5 yr.	6, 8, 12	105	Prompt reduction	Gradual improvement	Prompt disappearance	14 d.	Severe.	R. L.
Idem	14	S. M.	M.	11 yr.	8, 14, 15	90	Gradual reduction	Gradual improvement	.....	10 d.	Ordinarily severe.	R. L.
Idem	15	E. G.	F.	10 yr.	5, 6	50	Gradual reduction	Gradual improvement	.....	7 d.	Ordinarily severe. Deafness.	R. L.
Rhode Island Hospital, Providence	1	F. V.	M.	8 yr.	7, 8, 10, 32, 33, 35, 36, 37, 48, 49, 51, 52, 54, 56, 58, 60, 61, 63, 65, 67, 72, 75	655	.....	.....	.....	3-4 mo.	Severe. Two relapses with which reappearance of diplococci was associated. Very protracted course.	R. R.
Idem	2	T. D.	M.	9 yr.	6, 7, 8, 9	190	Gradual reduction	Gradual improvement	Rapid loss of viability	7 d.	Ordinarily severe.	R. L.

Idem	3	V. B.	M.	14 yr.	3, 5, 6, 7 8, 9, 10, 11, 12, 13, 15, 17, 20	390	Slow reduction	Slow improvement	Rapid loss of viability	24 d.	Severe.	R. L.
Idem	4	M. D.	F.	10 yr.	4, 5, 6, 8, 9	150	Prompt reduction	Prompt improvement	Rapid loss of viability	8 d.	Ordinarily severe. Deafness apparent on 2d day; persisted.	R. L.
Idem	5	R. E. B.	M.	15 yr.	10, 13	60	None	None	.....	.....	Severe. Death in 5th week.	D.
Idem	6	M. C.	F.	13 yr.	3, 4, 5	90	Gradual reduction	Gradual improvement	Rapid loss of viability	14 d.	Ordinarily severe.	R. L.
Idem	7	M. H.	M.	32 yr.	6, 7, 8	90	Gradual reduction	Gradual improvement	Rapid loss of viability	12 d.	Ordinarily severe.	R. L.
Idem	8	M. A. T.	F.	32 yr.	3, 4, 5, 6, 8	150	Gradual reduction	Gradual improvement	Rapid loss of viability	14 d.	Ordinarily severe.	R. L.
Idem	9	F. V.	M.	30 yr.	4, 5, 6, 7, 8, 9, 11	210	None	None	Rapid loss of viability	.....	Onset doubtful. Patient chronic alcoholic. Death on 15th day.	D.
Idem	10	C. L.	F.	6 yr.	4, 5, 6, 7, 9, 11	170	Gradual reduction	Gradual improvement	Rapid disappearance	10 d.	Ordinarily severe.	R. L.
Idem	11	L. S.	M.	16 yr.	5, 6, 7, 8, 10	150	Gradual reduction	Gradual improvement	Rapid disappearance	11 d.	Ordinarily severe.	R. L.
Idem	12	V. D.	F.	9 yr.	4, 5, 6, 7	120	Gradual reduction	Gradual improvement	.....	14 d.	Ordinarily severe.	R. L.
Idem	13	A. M.	M.	2 yr.	3 (?)	30	.....	.....	.....	.....	Moribund on admission. Died immediately after injection.	D. M.
Idem	14	F. A. A.	F.	51 yr.	6 (?)	30	.....	.....	.....	.....	Moribund on admissions. Died 12 hours after injection.	D. M.
Idem	15	R. S.	F.	14 yr.	4, 5, 6, 7	120	Rapid reduction	Rapid improvement	.....	4 d.	Mild.	R. C.
Idem	16	M. H.	F.	2 yr.	4	25	.....	.....	.....	.....	Comatose on injection. Death within 24 hours.	D. M.
Idem	17	L. G.	M.	16 yr.	10, 12, 13	75	.....	.....	.....	.....	History inadequate. Bacteriological diagnosis doubtful.	D.
Pennsylvania Hospital, Philadelphia	1	J. J.	M.	23 yr.	4	15	Rapid reduction	Rapid improvement	.....	4 d.	Ordinarily severe.	R. C.
Idem	2	R. B.	M.	17 yr.	3, 5	25	Gradual reduction	Gradual improvement	.....	5 d.	Ordinarily severe.	R. L.
Idem	3	A. G.	F.	15 yr.	4, 6, 9	45	Rapid reduction	Rapid improvement	Rapid disappearance	7 d.	Ordinarily severe.	R. C.
Idem	4	C. B.	M.	11 yr.	11, 12	25	None	None	.....	.....	Severe. Purulent exudate. Death on 13th day.	D.

TABLE I.—*Continued.*

Reported by	Case No.	Name	Sex	Age	Days of disease when serum injected	Total amount serum injected	Influence on temperature	Influence on special sympt.	Influence on Diplococci	Period of fever and sympt.	Type of disease and remarks	Result
Pennsylvania Hospital	5	P. C.	M.	18 yr.	10	15	Rapid reduction	Rapid improvement	.....	3 d.	Mild.	R. L.
Idem	6	A. L.	F.	8 yr.	6, 7	20	.....	None	.....	.....	Severe. Condition desperate at first injection. Death 48 hours later.	D.
Idem	7	W. H.	M.	19 yr.	5, 6	60	None	None	.....	.....	Ordinarily severe. Death on 8th day.	D.
Idem	8	M. E.	F.	4 yr.	4, 5	60	Rapid reduction	Gradual improvement	Rapid disappearance	9 d.	Severe.	R. L.
Idem	9	M. F.	F.	21 yr.	10, 11, 17, 18	120	Gradual reduction	Gradual improvement	Rapid disappearance	8 d.	Ordinarily severe.	R. L.
Idem	10	E. D.	M.	19 yr.	12 (?), 13	60	Rapid reduction	Rapid improvement	Rapid disappearance	4 d.	Mild.	R. C.
Garfield Hospital, Washington	1	M. S.	F.	18 yr.	10, 11, 12, 15, 17, 19, 21, 22	165	None	None	Rapid disappearance, reappearance and subsequent disappearance	48 d.	Ordinarily severe; protracted. Complicated by arthritis and choroiditis.	R. R.
Idem	2	F. L.	M.	5 mo.	49, ?	80	.....	.....	.....	.....	On admission chronic hydrocephalus and mixed diplococcus and bacillary infection.	D.* D.*
Idem	3	H. G.	M.	4 yr.	7, 13	80	Prompt reduction	Prompt improvement	Prompt disappearance	16 d.	Ordinarily severe.	R. L.
Idem	4	M. H.	F.	4 yr.	4	15	Gradual reduction	Gradual improvement	.....	11 d.	Severe. Deafness.	R. L.
Idem	5	I. O.	M.	25 yr.	8, 14	20	None	Gradual improvement	Rapid disappearance	23 d.	Ordinarily severe. Complicated by pneumonia. Mental impairment.	R. L.
Idem	6	J. J.	M.	16 yr.	34	15	Rapid reduction	Rapid improvement	.....	3 d.	Imperfect history of onset. Subacute. Abrupt termination after injection.	R. C.
Idem	7	W. B.	M.	30 yr.	4, 10, 12, 13, 20	105	None	None	None	.....	Ordinarily severe. Death on 25th day.	D.
Idem	8	E. N.	M.	3 yr.	8	15	.....	.....	Rapid disappearance	.....	Mild.	R. L.

Idem	9	T. L.	M.	17 yr.	14, 15, 17, 20, 21, 25	142.5	Gradual	Gradual	Prompt disappearance	20 d.	Ordinarily severe.	R. L.
Idem	10	W. W.	M.	23 yr.	4, 5 21, 25	45	Reduction	Prompt improvement	Rapid disappearance	.....	Severe. Complicated by pneumonia and paralysis of intestine. Death from peritonitis.	D.*
New York Hospital	1	S. D.	F.	18 mo.	15, 16, 17, 18, 19, 20, 21, 24, 25, 26	150	None	None	.....	.....	Insidious onset. Ordinarily severe. Death on 26th day.	D.
Idem	2	P. F.	F.	2.5 yr.	22, 23, 24, 25, 26, 27	90	Gradual reduction	Gradual improvement	.....	12 d.	Ordinarily severe.	R. L.
Idem	3	M. A. C.	F.	23 yr.	3, 4, 6, 14	135	Gradual reduction	Gradual improvement	.....	12 d.	Mild. Complicated with diphtheria.	R. L.
Idem	4	J. C.	M.	13 mo.	8, 9	50	.....	.....	.....	.....	Severe. Died within 24 hours of 1st injection.	D. M.
St. Luke's Hospital, N. Y.	1	J. B.	M.	29 yr.	20, 21	45	Slow reduction	Gradual improvement	.....	10 d.	Mild.	R. L.
Idem	2	M. S.	F.	14 yr.	4 (two)	45	None	None	.....	.....	Severe.	D.
S. Vincent's Hospital, N. Y.	1	A. C.	M.	22 yr.	?	30	None	None	.....	.....	Inadequate history. Condition desperate on admission. Death 3d day after injection.	D.
Idem	2	C. L.	M.	22 yr.	4	30	Prompt reduction	Prompt improvement	.....	8 d.	Severe.	R. L.
Idem	3	G. P.	M.	18 yr.	18 (?), 24	60	Prompt reduction	Prompt improvement	.....	27 d.	Ordinarily severe.	R. L.
Idem	4	A. L.	M.	11 yr.	34, 35	29.5	Rapid reduction	Rapid improvement	.....	2 d.	Mild. Protracted course.	R. C.
Presbyterian Hospital, N. Y.	1	D. O.	M.	2 yr.	6, 7, 8, 9, 12, 16, 23, 25, 26	155	None	None	Rapid reduction with subsequent increase.	.....	Ordinarily severe. Death on 26th day.	D.
Idem	2	G. R.	F.	4 yr.	3, 5, 6, 7, 8, 11, 15, 16, 17, 18, 19	295	Gradual reduction	Gradual improvement	Rapid loss of viability. Increase during relapse.	11 d.	Severe.	R. R.
Idem	3	F. D.	M.	2.5 yr.	16	30	Rapid reduction	Rapid improvement	.....	2 d.	Ordinarily severe.	R. C.
Idem	4	M. A.	F.	34 yr.	3, 5, 6, 7, 9, 10, 14	225	None	None	Slow reduction	.....	Severe. Intercurrent pericarditis. Death on 16th day.	D.
Idem	5	I. A.	M.	11 mo.	21, 25, 26, 27, 32	135	None	None	Prompt disappearance	.....	Semichronic with hydrocephalus. Intercurrent erysipelas. Death on 30th day.	D.*

TABLE I.—*Continued.*

Reported by	Case No.	Name	Sex	Age	Days of disease when serum injected	Total amount serum injected	Influence on temperature	Influence on special sympt.	Influence on Diplococci	Period of fever and sympt.	Type of disease and remarks	Result
Babies' Hospital, N. Y.	1	E. F.	F.	11 mo.	49 (?), 64, 75, 91, 112	47	None	None	Gradual disappearance	.....	Severe. Semi-chronic. Death on 119th day.	D.
Idem	2	S. H.	M.	6 mo.	23, 24, 25, 26, 27, 28, 29	60	Gradual reduction	Gradual improvement	Rapid disappearance	7 d.	Ordinarily severe.	R. L.
Idem	3	H. K.	M.	22 mo.	28, 29, 30, 31, 32, 33, 34, 35	117	Gradual reduction	Gradual improvement	.....	.....	Ordinarily severe.	R. L.
St. Mary's Hospital, N. Y.	1	H. F.	F.	4 yr.	20, 21	20	Rapid reduction	Rapid improvement	.....	3 d.	Mild.	R. C.
Idem	2	A. P.	M.	12 yr.	2, 3	40	Rapid reduction	Rapid improvement	Rapid disappearance	2 d.	Severe. Deaf on admission.	R. C.
Lakeside Hospital, Cleveland	1	B. K.	F.	16 yr.	3, 4, 19	25	Gradual reduction	Gradual improvement	.....	25 d.	Severe.	R. L.
Idem	2	F. W.	?	3 yr.	12	5	Rapid reduction	Rapid improvement	.....	?	Severe.	R. C.
Idem	3	J. B.	M.	23 yr.	4, 5, 6	30	Gradual reduction	Prompt improvement	.....	30 d.	Very severe.	R. L.
Idem	4	H.	M.	17 yr.	5	15	None	None	.....	.....	Severe with purulent exudate. Death within 24 hours of injection.	D. M.
Idem	5	W. F.	M.	3 yr.	2, 8, 23	35	Gradual reduction	Gradual improvement	.....	24 d.	Severe. Two relapses on 6th and 22d d. respectively.	R. R.
Idem	6	N.	F.	2 yr.	1	15	Rapid reduction	Rapid improvement	.....	6 d.	Severe.	R. C.
Idem	7	X.	F.	11 yr.	8, 9	20	Rapid reduction	Rapid improvement	.....	7 d.	Severe.	R. C.
Idem	8	W. H.	M.	21 yr.	3, 4, 7	40	Gradual reduction	Gradual improvement	.....	12 d.	Ordinarily severe.	R. L.
Idem	9	C. G.	F.	6 yr.	3, 4, 6	35	None	None	.....	.....	Severe. Death on 7th day.	D.
Idem	10	J. C.	M.	2 yr.	1	15	Rapid reduction	Rapid improvement	.....	6 d.	Severe.	R. C.
Idem	11	F. G.	M.	18 mo.	40 (?), 44	25	.....	.....	.....	.....	Semichronic. Hydrocephalus present. Death on 119th day.	D.
Idem	12	F. P.	M.	3 yr.	4, 5, 6	46	None	None	.....	.....	Severe. Death on 6th day.	D.
Idem	13	O. C.	F.	7 yr.	1, 2, 3	45	Rapid reduction	Rapid improvement	.....	4 d.	Severe.	R. C.

Idem	14	L. S.	M.	19 mo.	14, 17, 32, 45, 47	28	Gradual reduction	Slow improvement	.....	4 w.	Protracted course.	R. L.
Idem	15	C. D.	M.	8 yr.	3, 5, 8, 12, 14, 27	53	Slow reduction	Slow improvement	.....	4 w.	Ordinarily severe.	R. L.
Idem	16	I. L.	?	5 yr.	4, 5, 6, 7, 9, 19, 23	41	None	None	.....	.....	Ordinarily severe. Small serum injections. Hydrocephalus. Death on 45th day.	D.
Idem	17	P.	M.	10 yr.	90, 97	20	None	None	.....	.....	Hopelessly chronic. Death 5.5 mo. after onset.	D.*
Idem	18	A. C.	M.	6 mo.	? (5 injections given)	29	None	None	Rapid loss of viability	.....	Imperfect history. Purulent exudate. Subcutaneous injections. Hydrocephalus.	D.*
Idem	19	J. M.	M.	7 mo.	6, 7, 8, 16, 24, 26	60.5	Slow reduction	Slow improvement	Slow disappearance	4 w.	Ordinarily severe. Serum subcutaneous and subdural.	R. L.
Idem	20	H.	M.	7 yr.	4, 5, 12, 14	60	Gradual reduction	Gradual improvement	.....	15 d.	Ordinarily severe. Serum subcutaneous and subdural.	R. L.
Idem	21	R. H.	M.	15 yr.	4, 5, 6, 7, 8	73	Gradual reduction	Gradual improvement	Rapid loss of viability	11 d.	Ordinarily severe.	R. L.
Idem	22	C. L.	M.	8 yr.	2, 3	37	None	None	Culture negative after 1st injection	.....	Fulminant. Death on 3d day.	D. F.
Idem	23	R. R.	M.	21 yr.	21 (?), 24, 26, 27	36.5	.....	Gradual improvement	Rapid disappearance	10 d.	Ordinarily severe with normal temperature.	R. L.
Idem	24	E. S.	M.	6 yr.	5, 6, 7, 9	50	Gradual reduction	Gradual improvement	Rapid disappearance	10 d.	Ordinarily severe.	R. L.
Idem	25	A. H.	M.	15 mo.	5, 9, 13, 19	55	Gradual reduction	Prompt improvement	Rapid disappearance	14 d.	Severe.	R. L.
Idem	26	L.	M.	3 mo.	2d week (?) (9 injections?)	90(?)	None	None	Rapid disappearance	.....	Late case complicated by general oedema.	D.
Idem	27	R.	F.	16 yr.	3	20	.....	.....	.....	.....	Moribund. Purulent exudate. Survived injection 3 hours.	D. M.
Idem	28	J. D.	M.	23 yr.	3 (?) (two), 4 (two)	37.5	None	None	.....	.....	Very severe. Death on 5th day.	D.
Idem	29	V. M.	F.	12 yr.	2, 3	35	Rapid reduction	Rapid improvement	.....	2 d.	Severe.	R. C.
Akron City Hospital	1	V. H.	F.	9 yr.	7, 12, 15, 28, 37, 49	32.5	Gradual reduction	Gradual improvement	Rapid disappearance	59 d.	Ordinarily severe. Relapse on 48th day.	R. R.
Idem	2	H. R.	M.	17 yr.	2	10	None	None	.....	.....	Fulminant. Death 12 hours after injection.	D. F.

TABLE I.—*Continued.*

Reported by	Case No.	Name	Sex	Age	Days of disease when serum injected	Total amount serum injected	Influence on temperature	Influence on special sympt.	Influence on Diplococci	Period of fever and sympt.	Type of disease and remarks	Result
Akron City Hospital	3	B. S.	M.	19 yr.	15	20	Rapid reduction	Rapid improvement	.....	4 d.	Ordinarily severe.	R. C.
Idem	4	F. N.	M.	32 yr.	7	25	Rapid reduction	Rapid improvement	.....	3 d.	Ordinarily severe.	R. C.
Idem	5	B. K.	F.	15 yr.	2, 5, 7, 11	43.5	Slow reduction	Slow improvement	Prompt disappearance	23 d.	Ordinarily severe.	R. L.
Idem	6	Z. H.	F.	17 yr.	2, 4	22.5	Rapid reduction	Prompt improvement	.....	.....	Severe. Death occurred suddenly 2 hours after 3d injection on 4th day.	D.
Idem	7	H. S.	M.	14 yr.	1, 4	35	Rapid reduction	Rapid improvement	.....	10 d.	Severe.	R. C.
Idem	8	R. M.	M.	17 yr.	1	15	.....	.....	.....	.....	Fulminant. Death 30 hours after injection.	D. F.
Idem	9	G. G.	F.	17 yr.	1	22.5	Prompt reduction	Prompt improvement	.....	10 d.	Mild.	R. C.
Idem	10	J. A. S.	M.	24 yr.	2, 5, 11, 15	105	Slow reduction	Slow improvement	.....	17 d.	Ordinarily severe.	R. L.
Idem	11	E. R.	M.	20 yr.	1	25	Rapid reduction	Rapid improvement	.....	4 d.	Severe.	R. C.
Idem	12	H. B.	F.	6 yr.	6, 7, 8, 16, 29, 36	160	Slow reduction	Slow improvement	.....	49 d.	Severe.	R. L.
Montreal General Hospital	1	L. B.	F.	26 mo.	5, 6, 7, 17, 18	53	Gradual reduction	Gradual improvement	Gradual reduction, reappearance	.....	Ordinarily severe. First attack terminated on 7th day. Relapse and death on 46th day.	D. R.
Idem	2	M. F.	F.	6 yr.	2, 3, 4, 5, 6, 8	94	Prompt reduction	Prompt improvement	Rapid loss of viability	6 d.	Ordinarily severe.	R. L.
Idem	3	A. W.	F.	6 yr.	3, 5, 7, 8, 10, 12	170	Gradual reduction	Gradual improvement	.....	2 w.	Ordinarily severe.	R. L.
Idem	4	E. W.	F.	9 yr.	2, 3, 6, 7, 9, 10	153	Gradual reduction	Gradual improvement	Rapid loss of viability	2 w.	Ordinarily severe.	R. L.
Idem	5	J. L.	M.	9 yr.	2, 3, 8, 12, 13, 15	151	Gradual reduction	Gradual improvement	Rapid loss of viability	13 d.	Ordinarily severe.	R. L.
Idem	6	L. F.	M.	3 yr.	2	30	.....	.....	.....	.....	Severe. Death 6 hours after injection.	D. M.
Idem	7	F. L.	F.	10 yr.	3	30	.....	.....	.....	.....	Severe. Death one half hour after injection.	D. M.

Idem	8	S. C.	M.	39 yr.	2, 4	32.5	Prompt reduction	Prompt improvement	Diplococci not demonstrated	5 d.	Severe.	R. L.
Churchill, Chicago	1	R.	F.	2 yr.	16	30	.....	.....	.....	.....	Inadequate history. Death 2d day after injection.	D.
Idem	2	H. M.	M.	9 yr.	28 (?), 30, 33, 38, 39, 40, 43	120	Gradual reduction	Gradual improvement	Prompt disappearance	14 d.	Semichronic.	R. L.
Idem	3	M. B.	F.	4 yr.	2, 4	30	Rapid reduction	Rapid improvement	.....	6 d.	Severe.	R. C.
Idem	4	R. G.	M.	30 yr.	3d w. (1 injection)	30	Prompt reduction	Prompt improvement	Rapid disappearance	9 d.	Semichronic.	R. L.
Idem	5	F. J.	M.	14 yr.	3, 4, 6	90	Rapid reduction	Rapid improvement	Rapid disappearance	4 d.	Severe.	R. L.
Idem	6	J. B.	M.	16 yr.	7	30	Prompt reduction	Prompt improvement	Rapid disappearance	5 d.	Ordinarily severe.	R. L.
Idem	7	C. T.	M.	19 yr.	2, 4	55	Prompt reduction	Prompt improvement	Rapid disappearance	9 d.	Ordinarily severe.	R. L.
Idem	8	A. B.	F.	11 yr.	4, 5, 6	90	Prompt reduction	Prompt improvement	Rapid disappearance	9 d.	Ordinarily severe.	R. L.
Idem	9	R. S.	F.	14 yr.	12	15	.....	.....	.....	.....	Ordinarily severe. Moribund. Death 16 hours after injection.	D. M.
City Hospital, Cincinnati	1	A. M.	M.	19 yr.	16 (?), 17, 35, 42	120	.....	.....	.....	.....	Semichronic.	D.
Idem	2	G. D.	M.	18 yr.	25, 26	60	.....	.....	Rapid disappearance	.....	Semichronic. Emaciated and hopeless. Death on 28th day.	D.
Idem	3	C. T.	M.	18 yr.	2 (?), 3, 5, 6, 8, 9, 11, 12, 13, 15, 21, 24	315	.....	.....	Slow disappearance and loss of viability	.....	Severe.	R. L.
Idem	4	D. E.	M.	40 yr.	3	30	.....	.....	.....	.....	Very severe. Moribund.	D. M.
Idem	5	W. J.	M.	18 yr.	? (5 injections)	135	.....	.....	Prompt disappearance	.....	Inadequate history. Severe.	R. L.
Idem	6	T. R.	F.	4 yr.	3, 4, 7	45	Rapid reduction	Rapid improvement	.....	5 d.	Mild.	R. L.
Idem	7	K.	F.	2 yr.	3, 5	30	Rapid reduction	Rapid improvement	Rapid disappearance	5 d.	Ordinarily severe.	R. L.
Idem	8	V.	F.	10 yr.	3, 4, 7	90	Gradual reduction	Gradual improvement	Rapid disappearance	7 d.	Severe.	R. L.
Idem	9	K.	M.	11 mo.	2, 3	30	Gradual reduction	Gradual improvement	Rapid disappearance	6 d.	Ordinarily severe. Deafness.	R. L.
Idem	10	R. S.	M.	3 yr., 8 mo.	1	30	.....	.....	.....	.....	Fulminant. Death 4 hours after injection.	D. F.
Idem	11	A. K.	F.	7 yr.	1, 2	45	Rapid reduction	Rapid improvement	Rapid loss of viability	2 d.	Ordinarily severe.	R. C.

TABLE I.—*Continued.*

Reported by	Case No.	Name	Sex	Age	Days of disease when serum injected	Total amount serum injected	Influence on temperature	Influence on special sympt.	Influence on Diplococci	Period of fever and sympt.	Type of disease and remarks	Result
City Hospital, Cincinnati	12	T. K.	F.	4 wk.	2, 4	16	Gradual reduction	Gradual improvement	Rapid loss of viability	.....	Ordinarily severe.	R. L.
Idem	13	B. B.	M.	20 yr.	5, 6	60	.....	.....	.....	.....	Very severe.	D.
Idem	14	S.	F.	7 yr.	2, 3	60	Gradual reduction	Gradual improvement	Rapid loss of viability	5 d.	Ordinarily severe.	R. L.
Idem	15	G.	F.	10 yr.	3, 4	60	Prompt reduction	Prompt improvement	.....	5 d.	Ordinarily severe.	R. L.
Idem	16	C. O.	M.	15 yr.	1	30	.....	.....	.....	.....	Fulminant. Died within 24 hours of injection.	D. F.
Idem	17	A. J.	M.	20 yr.	20, 21	60	.....	.....	.....	.....	Semichronic. Purulent exudate.	D.
Idem	18	W. W.	F.	6 yr.	3, 4, 13, 19	60	Gradual reduction	Gradual improvement	.....	.....	Ordinarily severe.	R. R.
Idem	19	P. R.	M.	28 yr.	3, 4	60	.....	.....	.....	.....	Very severe. Purulent exudate. Death on 5th day.	D.
Idem	20	A. W.	M.	20 yr.	(?) (2 injections)	60	.....	.....	.....	.....	Inadequate history. Severe. Death soon after 2d injection.	D.
Idem	21	E. H.	M.	26 yr.	(?) (2 injections)	60	.....	.....	.....	.....	Severe. Death on the 12th day after second injection.	D.
Idem	22	H. R.	M.	4.5 yr.	2, 4, 7	45	None	None	.....	.....	Ordinarily severe.	D.
Idem	23	E. L.	M.	5 yr.	2, 4, 8, 11	105	Slow reduction	Slow improvement	.....	2 w.	Ordinarily severe.	R. L.
Idem	24	R.	M.	16 yr.	3, 4	60	Rapid reduction	Rapid improvement	.....	5 d.	Ordinarily severe.	R. L.
Idem	25	M. G.	F.	21 yr.	5, 7, 9	90	Gradual reduction	Gradual improvement	.....	.....	Ordinarily severe.	R. L.
Idem	26	A. E.	M.	17 yr.	3, 5, 7, 10	120	None	None	Rapid disappearance	.....	Ordinarily severe. Death on 14th day.	D.
Idem	27	A. H.	F.	25 yr.	3,4,6,10,13,15	180	None	.....	.....	.....	Ordinarily severe. Death on 22d day.	D.
Idem	28	E. G.	M.	16 yr.	6, 7, 9	90	.....	.....	.....	.....	Ordinarily severe.	R. L.
Idem	29	H. C.	F.	5 yr.	(?) (1 injection)	30	Rapid reduction	Rapid improvement	Diplococci not demonstrated	2 d.	Ordinarily severe.	R. C.
Idem	30	J. M.	M.	17 yr.	7, 10, 12, 14, 17	150	Slow reduction	Gradual improvement	Rapid disappearance	.....	Ordinarily severe.	R. L.
Idem	31	E. C.	M.	3 yr.	2, 3, 4	90	Prompt reduction	Prompt improvement	.....	8 d.	Ordinarily severe.	R. L.

Idem	32	K. B.	F.	12 yr.	2, 3, 4, 6, 9, 16, 17, 24	240	Slow reduction	Slow improvement	.....	.....	Ordinarily severe.	R. L.
Idem	33	M. B.	F.	8 yr.	1, 2, 4	75	Rapid reduction	Rapid improvement	Rapid disappearance	5 d.	Ordinarily severe. Early injection; clear exudate.	R. L.
Idem	34	C. B.	M.	2 yr.	1, 2, 3, 5	75	Rapid reduction	Rapid improvement	Prompt disappearance	5 d.	Ordinarily severe.	R. L.
Idem	35	M. B.	M.	2 yr.	5, 6, 8	60	Prompt reduction	Prompt improvement	.....	4 d.	Ordinarily severe.	R. L.
Idem	36	L. T.	M.	8 yr.	31 (?), 32, 33	90	Prompt reduction	Prompt improvement	.....	.....	Semichronic. Purulent exudate. Rapid clearing up of exudate.	R. L.
Idem	37	E. S.	M.	20 yr.	5	30	.....	.....	.....	.....	Moribund. Death 15 hours after injection.	D. M.
Idem	38	A. E.	M.	5 yr.	2, 4, 10, 11, 20	135	.....	.....	Prompt disappearance; reappearance during relapse	.....	Ordinarily severe. Two relapses.	R. R.
Idem	39	M. M.	F.	14 yr.	2, 3, 4	90	.....	.....	.....	.....	Ordinarily severe.	R. L.
Idem	40	C. G.	F.	14 yr.	2, 3, 4	90	.....	.....	.....	.....	Ordinarily severe.	R. L.
Idem	41	T. H.	M.	24 yr.	2, 3, 4, 5, 6, 8, 14	210	.....	.....	.....	.....	Ordinarily severe.	R. L.
Idem	42	J. H.	M.	18 yr.	5, 6, 7	90	.....	.....	.....	.....	Ordinarily severe.	R. L.
Idem	43	H. W.	M.	19 yr.	3, 13, 14, 17, 25	150	.....	.....	.....	.....	Ordinarily severe.	R. L.
Idem	44	A. D.	F.	5 yr.	3, 4, 5	90	.....	.....	.....	.....	Ordinarily severe.	R. L.
Idem	45	S.	F.	25 yr.	3, 4, 5, 7, 8, 13	150	.....	.....	.....	.....	Severe.	R. L.
Dunn, Boston	1	C. C.	M.	2 yr., 10 mo.	2, 3, 4, 5, 19, 20, 21, 22	181	Slow reduction	Slow improvement	Slow disappearance	23 d.	Ordinarily severe.	R. L.
Idem	2	B. K.	F.	3.5 yr.	8, 9, 10, 11, 19, 20, 21, 22	200	Slow reduction	Slow improvement	Rapid loss of viability	16 d.	Ordinarily severe.	R. L.
Idem	3	J. M.	M.	16 mo.	14, 15	60	None	None	.....	.....	Inadequate history. Death in 3d week.	D.
Idem	4	J. H.	M.	6.5 yr.	2, 3, 4, 5	120	Prompt reduction	Rapid improvement	Rapid loss of viability	6 d.	Severe.	R. C.
Idem	5	E. S.	F.	1 yr.	21, 22, 23, 24, 29, 30, 31, 32	295	Fall to normal after 1st injection	None	.....	.....	Death in 5th week.	D.
Idem	6	A. M.	M.	6 yr.	2, 3, 5	85	Rapid reduction	Rapid improvement	Rapid loss of viability	3 d.	Ordinarily severe.	R. C.
Idem	7	R. A.	M.	7 yr.	1	30	Rapid reduction	Rapid improvement	.....	4 d.	Mild.	R. C.
Idem	8	A. W.	F.	10 yr.	4, 5, 6	75	.....	Prompt improvement	Rapid loss of viability	5 d.	Ordinarily severe.	R. C.
Idem	9	W. W. W.	M.	6 yr.	1 (two)	60	Rapid reduction	Rapid improvement	.....	10 d.	Fulminant.	R. C.
Idem	10	A. R.	M.	4 yr.	2	30	Rapid reduction	Rapid improvement	.....	1 d.	Severe.	R. C.

TABLE I.—*Continued.*

Reported by	Case No.	Name	Sex	Age	Days of disease when serum injected	Total amount serum injected	Influence on temperature	Influence on special sympt.	Influence on Diplococci	Period of fever and sympt.	Type of disease and remarks	Result
Dunn, Boston	11	B. S.	F.	27 yr.	21, 22, 26, 32	165	.....	.....	Prompt reduction	.....	Severe course. Death in 5th week.	D.
Idem	12	E. D.	F.	14 wk.	7, 8, 12	75	Prompt reduction	Prompt improvement	.....	7 d.	Ordinarily severe.	R. L.
Idem	13	F. W.	M.	29 yr.	8, 9	60	Prompt reduction	Prompt improvement	Diplococci not demonstrated	4 d.	Mild.	R. L.
Idem	14	M. E. C.	F.	9 yr.	5, 6, 7, 8	120	Rapid reduction	Rapid improvement	Prompt disappearance	6 d.	Ordinarily severe.	R. C.
Idem	15	J. M.	F.	8 yr.	5, 6, 7, 8, 15, 18, 19, 20	275	Prompt reduction and relapse	Prompt improvement and relapse	Prompt reduction followed by increase during relapse.	15 d.	Ordinarily severe. Relapse on 5th day which endured 10 days.	R. R.
Idem	16	M. R. R.	F.	2 yr. 3 mo.	3, 4, 5	80	Gradual reduction	Gradual improvement	Prompt disappearance	7 d.	Severe.	R. L.
Idem	17	P. S.	F.	11 mo.	21, 23	60	.....	.....	.....	.....	Imperfect history. Blind and deaf on admission.	R. L.
Idem	18	M. B.	F.	16 yr.	20, 21, 23, 31	120	Gradual reduction	None	Prompt reduction	.....	Ordinarily severe. Death on 32d day.	D.
Idem	19	D. J. D.	M.	3.5 yr.	11, 12, 13, 14	140	Prompt reduction	Prompt improvement	.....	8 d.	Ordinarily severe.	R. L.
Idem	20	J. J. D.	M.	5 mo.	4, 5, 10, 12	45	Slow reduction	Slow improvement	.....	10 d.	Ordinarily severe.	R. L.
Idem	21	?	M.	13 yr.	28	30	None	None	.....	.....	Condition desperate at first injection. Death on 31st day.	D.
Idem	22	S. B.	F.	10 yr.	5, 6, 7, 9, 19	140	Gradual reduction	Gradual improvement	Prompt disappearance	6 d.	Ordinarily severe.	R. L.
Idem	23	J. D.	M.	4 yr.	56, 58	60	Prompt reduction	Prompt improvement	.....	8 d.	Protracted course; prompt recovery after serum injection.	R. L.
Idem	24	C. R.	F.	2 yr.	8	30	.....	Prompt improvement	.....	8 d.	Mild.	R. L.
Idem	25	J. F.	M.	2 yr.	3, 4, 5, 6	120	Gradual reduction	Gradual improvement	Rapid disappearance	14 d.	Severe.	R. L.
Idem	26	R. C.	M.	4.5 yr.	4, 5, 6, 7	120	Gradual reduction	Gradual improvement	Prompt disappearance	7 d.	Ordinarily severe.	R. L.
Idem	27	B. L.	F.	21 yr.	3, 4, 6, 7, 8, 9, 10 (after about 20th day 4 more injections)	330	.....	.....	.....	.....	Severe. Death in 4th week.	D. R.
Idem	28	A. H.	F.	5 yr.	3, 6, 4, 5, 11, 14, 23, 24, 25	280	Slow reduction	Slow improvement	Slow disappearance	24 d.	Severe. Deafness.	R. L.

Idem	29	L. C.	F.	9 yr.	3, 4, 5, 6, 7	115	Gradual reduction	Gradual improvement	Gradual disappearance	13 d.	Ordinarily severe.	R. L.
Idem	30	J. J. K.	M.	29 yr.	3 (two)	60	.....	.....	.....	.....	Fulminant. Death 4th day.	D. F.
Idem	31	W. M.	M.	16 yr.	6, 7, 8, 9	120	Rapid reduction	Rapid improvement	Rapid disappearance	3 d.	Mild.	R. C.
Idem	32	V. H.	M.	5 yr.	2, 4	45	Rapid reduction	Rapid improvement	Rapid disappearance	1 d.	Fulminant.	R. C.
Idem	33	A. T.	F.	24 yr.	21, 23	60	Rapid reduction	Rapid improvement	.....	1 d.	Protracted course. Immediate improvement after injection.	R. C.
Idem	34	G. L.	F.	7 yr.	8, 10	45	Rapid reduction	Rapid improvement	.....	1 d.	Ordinarily severe.	R. C.
Idem	35	T. F.	F.	4 yr.	5, 6, 7, 11	105	Rapid reduction	Rapid improvement	.....	7 d.	Mild.	R. C.
Idem	36	J. F. M.	M.	21 mo.	3, 4	60	Prompt reduction	Prompt improvement	.....	4 d.	Ordinarily severe.	R. L.
Idem	37			14 yr.	(?) (6 injections)	180	Gradual reduction	Gradual improvement	.....	10 d.	Ordinarily severe.	R. L.
Idem	38	M. O.	M.	15 yr.	6, 7	75	.....	.....	.....	.....	Collapse following serum injection: revived; died 29 hours later.	D.
Idem	39	A. A.	F.	2 yr., 7 mo.	35, 36, 38	45	.....	.....	.....	.....	Imperfect history. Died 7th week.	D.
Idem	40	C. B.	F.	4 yr.	? (8 injections)	205	Gradual reduction	Gradual improvement	Prompt disappearance	15 d.	Ordinarily severe.	R. L.
Robb, Belfast* (Purdysburn)	1	J. P.	M.	23 yr.	3, 6, 9	90	.....	.....	.....	7 d.	Ordinarily severe.	R. C.
Idem	2	R. J. B.	M.	8 mo.	23, 29	45	.....	.....	.....	.....	Semichronic. Purulent exudate. Death on 30th day.	D.
Idem	3	E. S.	F.	12 yr.	2, 4	60	.....	.....	.....	5 d.	Ordinarily severe.	R. L.
Idem	4	C. W.	F.	31 yr.	16, 19, 23	90	.....	.....	.....	14 d.	Severe.	R. L.
Idem	5	M. Q.	M.	19 yr.	3	30	.....	.....	.....	.....	Fulminant. Death 9 hours after injection.	D. F.
Idem	6	M. McG.	M.	22 yr.	8, 11	60	.....	.....	.....	4 w.	Ordinarily severe.	R. L.
Idem	7	J. C.	M.	20 yr.	6, 11, 23, 29, 52	210	.....	.....	.....	.....	Severe.	R. L.
Idem	8	P. H.	M.	13 yr.	2	30	.....	.....	.....	.....	Fulminant. Death 30 hours after injection.	D. F.
Idem	9	D. McI.	M.	11 yr.	7, 15, 18	75	.....	.....	.....	33 d. (?)	Ordinarily severe.	R. L.
Idem	10	J. B.	M.	19 yr.	6, 8, 13	80	.....	.....	.....	.....	Ordinarily severe.	R. L.
Idem	11	E. N.	F.	8 yr.	6, 13, 17, 19, 22, 34, 43	210	.....	.....	.....	.....	Severe. Purulent exudate.	R. L.
Idem	12	E. K.	F.	4 yr.	14, 17	60	.....	.....	.....	.....	Ordinarily severe.	R. L.
Idem	13	G. S.	M.	4 yr.	3	30	.....	.....	.....	.....	Fulminant. Purulent exudate. Death 6 hours after injection.	D. F.
Idem	14	C. S.	M.	13 yr.	2, 3	65	.....	.....	.....	4 d.	Fulminant onset.	R. C.
Idem	15	M. H.	M.	27 yr.	6, 7	60	.....	.....	.....	5 d.	Ordinarily severe.	R. L.

* Dr. Robb employed the serum at the two fever hospitals—Purdysburn and Union—in Belfast.

TABLE I.—*Continued.*

Reported by	Case No.	Name	Sex	Age	Days of disease when serum injected	Total amount serum injected	Influence on temperature	Influence on special sympt.	Influence on Diplococci	Period of fever and sympt.	Type of disease and remarks	Result
Robb, Belfast* (Purdysburn)	16	J. C.	F.	8 yr.	34, 36, 38, 44, 48, 52	180	.....	.....	.....	.....	Chronic. Death on 59th day.	D.
Idem	17	G. D.	F.	4 yr.	5, 6	60	.....	.....	.....	.....	Very severe. Purulent exudate. Death 3 hours after 2d injection.	D.
Idem	18	R. M.	F.	18 yr.	6, 7, 9, 11, 13, 15, 21, 26, 28, 32	315	.....	.....	.....	4 w.	Severe.	R. L.
Idem	19	S. S.	M.	11 yr.	2, 3, 5, 7, 9, 12, 15, 19, 24	290	.....	.....	.....	24 d.	Very severe. Purulent exudate.	R. L.
Idem	20	E. W.	F.	21 yr.	7, 13	60	.....	.....	.....	3 d.	Very severe.	R. C.
Idem	21	A. F.	F.	25 yr.	4 (two), 6, 7, 10, 13, 17, 21, 27	270	.....	.....	.....	33 d.	Very severe.	R. L.
Idem	22	H. G.	M.	17 yr.	1, 2, 4, 8	120	.....	.....	.....	11 d.	Very severe.	R. L.
Idem	23	S. J.	F.	4 yr.	5, 7	50	.....	.....	.....	.....	Ordinarily severe. Collapsed on admission.	D.
Idem	24	J. A.	F.	14 yr.	3, 5, 7, 11, 13, 19	180	.....	.....	.....	17 d.	Severe.	R. L.
Idem	25	R. B.	M.	27 yr.	32, 34, 38	120	.....	.....	.....	7 d.	Chronic.	R. L.
Idem	26	J. D.	M.	3 yr.	5, 6, 8, 11, 14, 16	180	.....	.....	.....	.....	Very severe. Collapsed for first four days after admission.	R. L.
Idem	27	A. McN.	F.	6 yr.	3, 4, 5, 6	100	.....	.....	.....	6 d.	Very severe.	R. L.
Idem	28	T. B.	M.	5 yr.	10, 11, 12, 14, 16, 18	180	.....	.....	.....	.....	Very severe. Collapsed on admission; never rallied.	D.
Idem	29	D. T.	M.	20 mo.	3, 4, 6, 9, 12	130	.....	.....	.....	9 d.	Ordinarily severe.	R. L.
Idem	30	M. D.	F.	15 mo.	7, 9, 15	120	.....	.....	.....	.....	Ordinarily severe. Death on 19th day.	D.
Idem	31	L. C.	F.	2 yr.	6, 8, 11	120	.....	.....	.....	6 d.	Ordinarily severe.	R. L.
Idem	32	B. S.	F.	2.5 yr.	3, 5	60	.....	.....	.....	7 d.	Ordinarily severe.	R. L.
Idem	33	R. McD.	M.	1 yr.	7, 16	60	.....	.....	.....	4 d.	Ordinarily severe. Relapse on 14th day. First attack 2 days; second 2 days.	R. R.
Idem	34	M. McC.	F.	4 yr.	4, 5	60	.....	.....	.....	.....	Collapsed on admission. Death on 7th day.	D.

Idem	35	F. A.	M.	23 yr.	5	30	.....	.....	.....	.....	Moribund on admission. Death half hour after injection.	D. M.
Idem	36	W. D.	M.	26 yr.	2 (two), 7	100	.....	.....	.....	7 d.	Ordinarily severe. Purulent exudate.	R. L.
Idem	37	M. H.	F.	5 yr.	5, 7	60	.....	.....	.....	5 d.	Ordinarily severe. Purulent exudate.	R. C.
Idem	38	W. R.	M.	9 yr.	15	30	.....	.....	.....	3 d.	Mild.	R. C.
Idem	39	L. H.	F.	13 yr.	2, 7	60	.....	.....	.....	7 d.	Ordinarily severe.	R. L.
Idem	40	R. D.	M.	8 yr.	3	30	.....	.....	.....	3 d.	Mild.	R. C.
Idem	41	S. McG.	F.	4 yr.	5	30	.....	.....	.....	3 d.	Ordinarily severe.	R. C.
Idem	42	T. S.	M.	3 mo.	5, 8, 17, 20, 25, 26	150	.....	.....	.....	3 w.	Ordinarily severe. Purulent exudate.	R. L.
Idem	43	A. S.	F.	2 yr.	5, 8	60	.....	.....	.....	6 d.	Ordinarily severe. Purulent exudate.	R. L.
Idem	44	J. B.	M.	43 yr.	2	45	.....	.....	.....	.....	Fulminant. Purulent exudate. Death 20 hours after injection.	D. F.
Idem	45	J. F. M.	M.	8 yr.	2, 3, 4, 6, 7, 10	180	.....	.....	.....	18 d.	Very severe. Purulent exudate.	R. L.
Idem	46	A. D.	M.	6 yr.	3, 4	45	.....	.....	.....	.....	Severe. Purulent exudate. Collapsed on admission. Death on 4th day.	D.
Idem	47	F. R.	M.	21 yr.	2, 3, 4, 5, 7, 9	195	.....	.....	.....	16 d.	Severe.	R. L.
Idem	48	L. McC.	F.	5 yr.	1, 3, 4, 9	120	.....	.....	.....	13 d.	Ordinarily severe. Purulent exudate.	R. L.
Idem	49	A. I.	F.	2.5 yr.	4, 6, 7	67	.....	.....	.....	.....	Ordinarily severe. Cyanosed on admission. Death on 8th day.	D.
Idem	50	H. F.	F.	2 yr., 9 mo.	75	30	.....	.....	.....	.....	Chronic.	R. L.
Idem	51	J. L.	F.	4.5 yr.	4	30	.....	.....	.....	.....	Cyanosed on admission. Death 7 hours after injection.	D. M.
Idem	52	S. F.	M.	20 yr.	10, 12, 16	90	.....	.....	.....	7 d.	Ordinarily severe.	R. L.
Idem	53	A. A.	F.	9 mo.	3, 5, 9, 13	90	.....	.....	.....	15 d.	Ordinarily severe.	R. L.
Idem	54	E. K.	F.	4 yr.	1, 3, 4	75	.....	.....	.....	.....	Ordinarily severe. Death on 4th day.	D.
Idem	55	W. W.	M.	14 yr.	2, 4, 6, 8, 11	150	.....	.....	.....	.....	Ordinarily severe.	R. L.
Robb, Belfast (Union)	56(1)	R. C.	M.	14 yr.	35	30	.....	.....	.....	.....	Semichronic.	R. L.
Idem	57(2)	F. McC.	M.	17 yr.	25, 32	60	.....	.....	.....	.....	Semichronic.	R. L.
Idem	58(3)	P. O'H.	M.	40 yr.	52	30	.....	.....	.....	.....	Semichronic.	R. L.
Idem	59(4)	W. H. G.	M.	29 yr.	57	30	.....	.....	.....	.....	Semichronic.	R. L.
Idem	60(5)	T. D.	M.	14 yr.	4, 10	60	.....	.....	.....	.....	Ordinarily severe. Death on 11th day.	D.
Idem	61(6)	M. K.	F.	21 yr.	7	30	.....	.....	.....	3 d.	Ordinarily severe.	R. C.

TABLE I.—*Continued.*

Reported by	Case No.	Name	Sex	Age	Days of disease when serum injected	Total amount serum injected	Influence on temperature	Influence on special sympt.	Influence on Diplococci	Period of fever and sympt.	Type of disease and remarks	Result
Robb, Belfast (Union)	62(7)	S. K.	M.	20 yr.	3, 11	60	.....	.....	.....	.....	Very severe. Deafness.	R. L.
Idem	63(8)	H. J.	M.	24 yr.	16, 24	60	.....	.....	.....	.....	Ordinarily severe.	R. L.
Idem	64(9)	M. McC.	F.	3 yr.	6, 10, 15	75	.....	.....	.....	.....	Very severe. Death on 15th day.	D.
Idem	65(10)	D. S.	M.	7 yr.	8	30	.....	.....	.....	4 d.	Mild.	R. L.
Idem	66(11)	S. D.	F.	14 yr.	29, 40	60	.....	.....	.....	.....	Semichronic. Intermittent.	R. L.
Idem	67(12)	F. B.	M.	5 yr.	7	30	.....	.....	.....	2 d.	Mild.	R. C.
Idem	68(13)	S. G.	F.	22 yr.	13, 17	60	.....	.....	.....	6 d.	Severe.	R. C.
Idem	69(14)	H. W.	M.	10 yr.	2, 3, 6, 10	120	.....	.....	.....	13 d.	Very severe.	R. C.
Idem	70(15)	J. L.	M.	36 yr.	12	30	.....	.....	.....	.....	Very severe. Death 30 hours after injection.	D.
Idem	71(16)	R. G.	M.	21 yr.	11, 12	60	.....	.....	.....	5 d.	Severe.	R. C.
McCantrie, Woolwich, England	1	C. M. M.	M.	19 yr.	4	30	Rapid reduction	Rapid improvement	.....	2 d.	Severe.	R. C.
Hebrew Hospital, Baltimore	1	L. A.	M.	7 yr.	1,2,3,4,6,8,9, 11, 13, 15, 16	225	Slow reduction	Slow improvement	.....	3-4 w.	Severe. Protracted course.	R. L.
Idem	2	F. B.	F.	20 yr.	6, 7, 8, 11	120	Gradual reduction	Gradual improvement	Rapid disappearance	8 d.	Severe.	R. L.
Idem	3	J. C.	M.	5 yr.	28, 29, 30, 31, 32, 33	180	Slow reduction	Slow improvement	.....	26 d.	Ordinarily severe.	R. L.
Morse, Boston	1	R. B.	M.	2 yr.	(7) (3 injections)	40	.....	.....	.....	.....	Chronic. Uncertain duration.	D.
Idem	2	C.K.	F.	28 yr.	10	25	Rapid reduction	Rapid improvement	.....	2 d.	Ordinarily severe.	R. C.
Idem	3	I. R.	F.	1 yr.	14, 15, 16, 17	57	Prompt reduction	Prompt improvement	Rapid disappearance	5 d.	Semichronic.	R. L.
Idem	4	M. S.	F.	4 mo.	42, 43, 44, 45, 46, 47, 48, 50, 52, 53, 54, 56, 57, 58, 59	184	.....	.....	.....	.....	Chronic. Only 1st puncture yielded fluid.	D.
Boston City Hospital	1	G. W. M.	M	28 yr.	2, 3, 4	85	.....	.....	.....	.....	Fulminant. Death on 4th day.	D. F.
Idem	2	L. M.	F.	32 yr.	6, 7, 8	70	Prompt reduction	Prompt improvement	Rapid disappearance	10 d.	Ordinarily severe.	R. L.
Idem	3	J. M.	F.	48 yr.	13, 14, 19	85	None	None	.....	.....	Ordinarily severe. Complicated with pneumonia. Death on 26th day.	D.

Idem	4	N. E.	M.	14 yr.	4, 5, 6, 7, 10, 11, 13, 16	235	Gradual reduction	Gradual improvement	.....	15 d.	Ordinarily severe.	R. L.
Idem	5	O. H.	M.	28 yr.	6 injections	180	.....	.....	.....	.....	No history. Ordinarily severe.	R. L.
Idem	6	M. A.	F.	11 yr.	7, 19	30	Rapid reduction	Rapid improvement	.....	5 d.	Ordinarily severe. 1st injection doubtfully intraspinal.	R. C.
Idem	7	E. L.	F.	35 yr.	20 (?), 23	45	Prompt reduction	Prompt improvement	.....	7 d.	Mild.	R. L.
Idem	8	M. N.	F.	14 yr.	3, 5	40	Rapid reduction	Rapid improvement	Rapid disappearance	5 d.	Ordinarily severe.	R. C.
Idem	9	J. O'N.	M.	24 yr.	13, 14	60	.....	.....	.....	.....	Severe.	D.
Idem	10	M. M.	M.	4 yr.	(?) (2 injections)	45	Rapid reduction	Rapid improvement	Diplococci not demonstrated	.....	Ordinarily severe.	R. C.
Massachusetts Gen. Hospital	1	E. T.	M.	8 yr.	2, 3, 4, 5, 9, 10, 11	195	Gradual reduction	Gradual improvement	.....	21 d.	Severe. Deafness.	R. L.
Idem	2	L. D.	M.	8 yr.	3	30	.....	.....	.....	.....	Severe. Death within 24 hours of injection.	D. M.
Portersville, California	1	F. H.	M.	14 mo.	2, 3, 4, 5, 11, 12, 13	127.5	Gradual reduction	Gradual improvement	.....	12 d.	Ordinarily severe.	R. L.
Idem	2	L. E. R.	M.	8 yr.	1, 2	45	Rapid reduction	Rapid improvement	.....	3 d.	Ordinarily severe.	R. C.
Idem	3	F. G.	F.	19 yr.	2, 3, 4	75	Prompt reduction	Prompt improvement	.....	.....	Ordinarily severe. Sudden return of severe symptoms. Death on 5th day	D.
Idem	4	M. S.	F.	6 yr.	1, 2, 3, 4	120	Prompt reduction	Prompt improvement	Rapid disappearance	4 d.	Ordinarily severe.	R. L.
Clayton, California	1	G. B.	M.	22 yr.	21, 22, 23, 24	120	Prompt reduction	Prompt improvement	.....	5 d.	Semichronic.	R. L.
Newark City Hospital	1	G. S.	M.	7 yr.	21, 23, 24, 25, 28, 29, 30, 31	134.5	Slow reduction	Slow improvement	Slow disappearance	.....	Semichronic.	R. L.
Newark	2	M. S.	M.	6 mo.	60, 64, 82, 88	60	None	None	.....	.....	Chronic. Clear spinal fluid. Death on 93d day.	D.*
McCulloch, Seattle	1	R. L.	M.	15 yr.	2, 3, 4	90	Rapid reduction	Rapid improvement	.....	3 d.	Severe.	R. C.
Idem	2	H.	?	child	3 (two)	18	.....	.....	.....	.....	Severe. Death within 24 hours of injection.	D. M.
Idem	3	J. H.	M.	30 yr.	(?) (5 injections)	150	.....	.....	.....	.....	Patient found unconscious. No history.	D.
Idem	4	W. O.	M.	4 yr.	1, 2	23	.....	.....	.....	.....	Fulminant. Death 24 hours after injection.	D. F.
Herzog, Chicago	1	?	M.	17 yr.	21, 22, 23, 24, 25, 27, 31, 32	210	Gradual reduction	Gradual improvement	Rapid disappearance	.....	Semichronic.	R. L.
Idem	2	F. R.	M.	23 yr.	7, 8, 9, 13, 14	105	Prompt reduction	Prompt improvement	.....	.....	Ordinarily severe.	R. L.
Adams, Washington	1	F. T.	M.	5 yr.	2, 4, 5, 7, 11, 13, 15, 17, 20	150	None	None	Diminished but not removed	.....	Severe. Death on 20th day.	D.

TABLE I.—*Continued.*

Reported by	Case No.	Name	Sex	Age	Days of disease when serum injected	Total amount serum injected	Influence on temperature	Influence on special sympt.	Influence on Diplococci	Period of fever and sympt.	Type of disease and remarks	Result
Adams, Washington	2	R. C.	M.	13 yr.	4, 5	45	Prompt reduction	Prompt improvement	.....	6 d.	Severe. 1st injection subcutaneous. No effect. 2d subdural.	R. L.
Idem	3	C. V. C.	M.	7 mo.	7, 14	30	Gradual reduction	Gradual improvement	.....	8 d.	Ordinarily severe.	R. L.
Raymond, Columbus, Ohio	1	C. F. G.	M.	adult	5, 6	60	Rapid reduction	Rapid improvement	.....	2 d.	Ordinarily severe.	R. C.
Idem	2	A. N. L.	M.	adult	10, 12	60	.....	.....	.....	.....	Very severe. Death on 13th day.	D.
Idem	3	G. B.	M.	23 yr.	1, 2	60	.....	.....	.....	.....	Fulminant. Death on 2d day.	D. F.
Idem	4	C. C. C.	M.	38 yr.	1, 2, 3	45	Rapid reduction	Rapid improvement	.....	3 d.	Ordinarily severe.	R. C.
Idem	5	C. W. C.	M.	adult	1, 2, 3, 4	60	None	None	.....	.....	Very severe.	D.
Naval Hospital, Newport	1	F. F. C.	M.	17 yr.	28,30,31,33,34	150	Slow reduction	Slow improvement	.....	.....	Chronic with apparent recovery. Sudden death from hydrocephalus, on 130th day.	D.
Idem	2	R. B. H.	M.	19 yr.	8, 9, 10, 13	115	Prompt reduction	Prompt improvement	Rapid disappearance	7 d.	Ordinarily severe.	R. L.
Idem	3	G. H. G.	M.	17 yr.	2,3,4,5,7,17, 19,20,21,22,24	330	None	Improvement with relapse	Slow reduction	.....	Severe. Autopsy showed no purulent exudate in meninges, but excessive quantity of clear fluid in lateral ventricles. Complicating pneumonia.	D.
Nichols, Washington	1	J. S.	M.	53 yr.	4	25	.....	.....	.....	.....	Severe. Death within 24 hours of injection.	D. M.
Idem	2	P. W.	M.	18 yr.	6, 8	60	.....	.....	.....	.....	Severe. Serum injected together with magnesium sulphate.	D.
Idem	3	C. H.	M.	22 yr.	14, 16, 18, 23	120	.....	.....	.....	.....	Ordinarily severe. Death on 25th day.	D.
Gouveneur Hospital, N. Y.	1	P. McG.	M.	23 yr.	3, 4, 5, 6	120	.....	.....	.....	.....	Severe. Only one injection certainly entered canal.	D.

Idem	2	M. F. I.	M.	18 yr.	3 (9 injections)	?	.....	.....	.....	.....	Ordinarily severe.	R. L.
Kingston Ave. Hospital, Brooklyn	1	W. O.	M.	3 yr.	16,17,20,23,25	65	None	None	.....	.....	Ordinarily severe.	D.
Idem	2	Y. C.	F.	7 yr.	12, 13, 20	63	Gradual reduction	Gradual improvement	.....	10 d.	Ordinarily severe.	R. L.
Roosevelt Hospital, N. Y.	1	J. M.	M.	adult	8, 10	50	Prompt reduction	Prompt improvement	Rapid disappearance	10 d.	Ordinarily severe.	R. L.
Bellevue Hospital, N. Y.	1	M. S.	M.	5 mo.	11, 12, 13, 14, 15, 16	108	.....	.....	.....	.....	Ordinarily severe. Improvement until 4th day, after 3d injection; then secondary streptococcus infection. Death on 17th day.	D.*
Hartford Hospital, Connecticut	1	J. J. B.	M.	42 yr.	22, 23, 25, 27	105	Prompt reduction	Prompt improvement	.....	.....	Semichronic. Death on 37th day from cardiac disease.	D.
Royal Victoria, Montreal	1	R. C.	M.	8 yr.	5, 6, 7, 8,10, 12,14,16,19,24	260	None	None	At first reduction, then increase	.....	Severe. Death on 25th day.	D.
University Hospital, Baltimore	1	L. D.	F.	21 yr.	12,13,14,15,16	67	.....	.....	.....	.....	Ordinarily severe. Death on 17th day.	D.
Mt. Sinai Hospital, Philadelphia	1	S. S.	M.	28 yr.	28, 36	60	.....	Prompt improvement	.....	.....	Ordinarily severe.	R. L.
Jewish Hospital, Brooklyn	1	J. S.	F.	6 yr.	4, 5, 6, 7, 8, 9, 11, 13, 14	270	Gradual reduction	Gradual improvement	Rapid disappearance	12 d.	Mild.	R. L.
German Hospital, N. Y.	1	R. K.	F.	11 yr.	5, 6	25	Rapid reduction	Rapid improvement	Rapid disappearance	5 d.	Ordinarily severe.	R. C.
Yorkville Hospital, N. Y.	1	E. H.	F.	16 mo.	21 (?)	15	.....	.....	.....	.....	Moribund. Death within 24 hours of injection.	D. M.
Sydenham Hospital, N. Y.	1	J. P.	F.	1 yr.	56, 57, 58, 59	60	None	None	.....	.....	Chronic.	D.
Hunt, Worcester	1	J. C.	M.	3.5 yr.	6, 7	30	Rapid reduction	Rapid improvement	.....	3 d.	Mild.	R. L.
Jennings, New York	1	E. B.	F.	4 mo.	21, 22, 23, 24, 26,27,28,30,32	134	.....	.....	.....	.....	Ordinarily severe. After 6th injection no fluid by puncture. Death from hydrocephalus on 54th day.	D.
Read, Brooklyn	1	?	M.	8 yr.	3, 4	60	.....	Prompt improvement	.....	5 d.	Ordinarily severe.	R. L.
Snowden, Peekskill, N.Y.	1	M.	M.	38 yr.	11, 12, 13	88	.....	.....	Rapid disappearance	.....	Severe. Death on 15th day.	D.

TABLE I.—*Continued.*

Reported by	Case No.	Name	Sex	Age	Days of disease when serum injected	Total amount serum injected	Influence on temperature	Influence on special sympt.	Influence on Diplococci	Period of fever and sympt.	Type of disease and remarks	Result
Williams, Rochester	1	?	M.	adult	2	15	.....	.....	.....	.....	Severe. Death 12 hours after injection.	D. M.
Teeter, Newark	1	C. J.	M.	12 yr.	8, 10, 11, 12, 13, 14, 15	165	Gradual reduction	Gradual improvement	Gradual disappearance	.....	Severe. Purulent exudate with improvement and sudden death in 5th week from hydrocephalus.	D.
Harris, St. Louis	1	A. E.	F.	23 yr.	4, 5, 6, 10, 13, 15, 20	144	None	None	.....	.....	Severe. Complicated with pregnancy and abortion. Purulent exudate. Death on 23d day.	D.
Diefendorf, New Haven	1	A.	M.	12 yr.	8, 10, 13, 18	135	Gradual reduction	Gradual improvement	.....	12 d.	Ordinarily severe.	R. L.
Flexner, Louisville	1	Z.	F.	16 yr.	6, 7, 14, 15, 16	150	Slow reduction	Slow improvement	.....	.....	Ordinarily severe.	R. L.
Idem	2	R.	M.	14 mo.	16, 17, 18	60	.....	.....	.....	.....	Ordinarily severe.	D.
Mohr, Ottawa, Canada	1	M. McD.	F.	15 yr.	15, 17, 19, 20, 34, 36	150	Gradual reduction	Gradual improvement	Prompt disappearance	.....	Ordinarily severe. Purulent exudate.	R. L.
Ker, Edinburgh	1	C. S.	M.	4 yr.	5, 6, 7	90	Gradual reduction	Gradual improvement	.....	10 d.	Ordinarily severe. Deafness.	R. L.
Idem	2	A. M.	F.	3 yr.	4, 5, 6, 8	120	Gradual reduction	Gradual improvement	.....	.....	Ordinarily severe.	R. L.
Idem	3	M. Q.	M.	18 yr.	9, 10, 11, 12, 17	150	Gradual reduction	Gradual improvement	.....	10 d.	Ordinarily severe.	R. L.
Idem	4	C. D.	F.	7 yr.	2	30	.....	.....	.....	.....	Fulminant. Death within 24 hours of injection.	D. F.
Idem	5	E. M.	F.	21 yr.	6	30	.....	.....	.....	.....	Severe. Complicated by mitral disease. Death 9 hours after injection.	D. M.
Idem	6	T. S.	M.	7 yr.	4, 5	60	None	None	.....	.....	Severe. Purulent exudate. Death on on 12th day.	D.
Idem	7	E. F.	F.	19 yr.	2, 3, 4	85	None	None	.....	.....	Very severe. Death on 6th day.	D.
Idem	8	P. W.	M.	30 yr.	5, 6, 7	85	None	None	.....	.....	Very severe. Purulent exudate. Death on 8th day.	D.

Idem	9	W. B.	M.	13 yr.	2, 3, 4, 6, 8, 12, 17	210	.....	.....	.....	.....	Ordinarily severe. Relapses. First attack 7 days. Second attack 3 days. Death on 19th day.	D. R.
Idem	10	C. G.	F.	4 yr.	2, 3, 4	45	Rapid reduction	Rapid improvement	Rapid disappearance	.....	Ordinarily severe.	R. C.
Idem	11	D. C.	M.	10 mo.	5, 6, 7, 8, 9	75	None	None	.....	.....	Very severe. Death on 12th day.	D.
Idem	12	A. M.	M.	12 yr.	12, 13, 14, 15	120	Rapid reduction followed by relapse	Gradual improvement followed by relapse	.....	.....	Ordinarily severe. Relapse on 7th day. Death on 18th day.	D. R.
Idem	13	M. P.	F.	48 yr.	4, 5	60	Prompt reduction	Prompt improvement followed by relapse	.....	.....	Ordinarily severe. Death on 20th day.	D. R.
Idem	14	J. G.	M.	14 yr.	8, 9, 11, 12, 14, 15, 25, 27, 37, 38, 58, 59, 60, 62	360	None	None	.....	.....	Severe.	D. R.
Idem	15	F. B.	F.	4 yr.	6, 7, 8, 9, 12	105	Gradual reduction	Gradual improvement	.....	.....	Severe.	R. C.
Idem	16	J. S.	F.	5 yr.	2, 3, 5, 6, 8, 10	150	Gradual reduction	Gradual improvement	Prompt reduction	.....	Very severe.	R. L.
Idem	17	R. S.	M.	5 yr.	5, 6, 8, 10, 13	145	Prompt reduction	Prompt improvement	Prompt reduction	.....	Severe.	R. R.
Idem	18	A. S.	F.	6 yr.	6, 7	60	.....	Rapid improvement	.....	.....	Ordinarily severe. Normal temperature.	R. C.
Idem	19	H. S. N.	F.	2 yr.	4, 5	60	Rapid reduction	Rapid improvement	.....	.....	Mild.	R. C.
Idem	20	J. R.	M.	17 yr.	2 (two), 4, 5	120	Rapid reduction	Rapid improvement	Rapid reduction	.....	Severe. Purulent exudate.	R. L.
Idem	21	P. P.	M.	22 yr.	7, 8, 9, 12, 14, 16	210	None	None	.....	.....	Very severe.	D.
Idem	22	R. B.	M.	2 yr.	5, 6, 7, 13, 14	120	Prompt reduction	Prompt improvement	Rapid disappearance	.....	Ordinarily severe.	R. R.
Idem	23	G. D.	M.	26 yr.	5, 6, 7	120	Prompt reduction	Prompt improvement	.....	.....	Severe.	R. C.
Idem	24	E. M.	F.	17 yr.	5	15	.....	.....	.....	.....	Severe. Moribund. Death 3 hours after 1st injection.	D. M.
Idem	25	S. McG.	F.	12 yr.	4, 5, 6	90	Prompt reduction	Prompt improvement	.....	.....	Ordinarily severe.	R. L.
Idem	26	J.W.McC.	M.	2 yr.	4, 5, 8, 9	120	.....	Prompt improvement	.....	.....	Ordinarily severe. Normal temperature.	R. R.
Idem	27	J. McD.	F.	1 yr.	7, 8, 9, 11, 13, 16, 17	195	Gradual reduction	Gradual improvement	.....	.....	Mild.	R. L.
McQueen, Ayrshire	1	?	M.	6 yr.	? (two injections)	60	Rapid reduction	Rapid improvement	.....	.....	Ordinarily severe.	R. C.

THE RELATION OF LESIONS OF THE ADRENAL GLAND TO CHRONIC NEPHRITIS AND TO ARTERIOSCLEROSIS; AN ANATOMICAL STUDY.[1]

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This investigation has for its object the determination, as far as may be possible by anatomical study, of the relation which exists between arteriosclerosis and changes in the adrenal gland on the one hand, and between chronic interstitial nephritis and adrenal lesions on the other.

The problem is of considerable general interest at the present moment in view of the numerous recent publications of French clinicians on this subject and has attracted my attention in connection with a recent critical study of the theory of chemical correlation as applied to the kidney.[2] It would appear natural, in view of our knowledge of the pressor effect of adrenalin and of the experimental lesions produced in the rabbit by this substance, as well as of our knowledge of the frequent association of hypertension with chronic interstitial nephritis and arteriosclerosis, to associate both the renal and arterial disturbances with some alteration of the adrenal. These suggestive facts, taken in connection with the rapidly accumulating evidence of intimate chemical correlation between widely separated organs, renders the problem an exceedingly interesting and suggestive one.

Within the past three years French investigators, led by Vaquez, have contributed a large mass of literature which indicates that localized or diffuse hyperplasia of the adrenal is commonly associated, when the disease does not run too rapid a course, with con-

[1] Work aided by a grant from the Rockefeller Institute for Medical Research. Received for publication July 15, 1908.

[2] Pearce, R. M., The Theory of Chemical Correlation as Applied to the Pathology of the Kidney. Annual address before the Philadelphia Pathological Society, April 23, 1908.

tracted kidney and arteriosclerosis. The hyperplasia is considered as an indication of hyperactivity of the antitoxic and angiotonic functions of the gland, what we might call perhaps hyperadrenalism.

The literature of this subject is now so voluminous that it would be unwise to review it completely, especially as the anatomical studies have been recently very completely collected and described by Rose,[3] and the somewhat scanty and not very convincing experimental studies have been similarly treated by Darré.[4] To indicate, however, the observations upon which this theory is based a portion of the earlier literature should be given briefly:

The first case described by Vaquez was one of adenoma of the cortex of the adrenal associated with a contracted kidney. Josué described three instances of diffuse arteriosclerosis with hypertrophy of the adrenal. Aubertin and Ambard studied eight cases in which there was contracted kidney and found fatty adenoma in three, diffuse hyperplasia in four and in the remaining case with a rapid clinical course a normal adrenal. Lemaire in a single instance, and Froin and Rivet in six out of seven cases of nephritis found adenomata or nodular hyperplasia; the remaining patient, in whom the rise in blood pressure was slight, had a normal adrenal. Menetrier found two adenomata in seven patients with contracted kidney. These figures indicate the frequency with which pathological changes have been described in the adrenals in association with renal and vascular lesions. There are many negative findings however and the frequency of similar lesions with diseases other than those of the kidney and the vascular system have not been sufficiently investigated. On the other hand Landau[5] who has examined the adrenals of sixteen individuals, suffering from general arteriosclerosis between the ages of forty-nine and ninety-six years found no changes which might not be ascribed to the effect on the gland of arteriosclerosis of the vessels of the adrenal itself.

In the hope of throwing more light on the subject by purely anatomical studies I have examined the autopsy records of the Bender Laboratory and have attempted to determine the relation of vascular and nephritic lesions to changes in the adrenal. Attention has been paid also to the occurrence of hyperplasia of the adrenal in association with conditions other than chronic vascular or renal disease.

[3] Rose, F., Blutdrucksteigerung, Schrumpfniere und Nebennieren, *Med. Klinik,* 1907, iii, 1405.

[4] Darré, H., De l'influence des altérations du rein sur les glandes surrénales, Paris, 1907.

[5] Landau, H., Ueber die anatomischen Veränderungen in den Nebennieren bei Arteriosklerose, *Zeitschr. f. klin. Med.,* 1907, lxiv, 227.

It has not been an easy matter in all instances, on account of the slighter variations so common in the adrenal, to establish a dividing line between a "normal picture" and the changes in the gland considered by the French writers to be characteristic of chronic interstitial nephritis. An arbitrary normal standard was finally established by comparing the descriptions of several histologists with sections of the adrenal of twelve individuals between the ages of forty and fifty-six years who had met death by violence and were free from chronic disease of any kind, and whose glands were therefore presumably normal for the ages given. The validity of the establishment of this normal picture was further strengthened by the study of glands of forty-six individuals under thirty years of age dying as the result of various acute infectious diseases. In this way the more acute degenerative changes were controlled.

On the other hand the descriptions of Aubertin and Ambard[6] and of Vaquez and Aubertin[7] have been taken as depicting accurately the changes deemed by the French writers to be characteristic of chronic interstitial nephritis with hypertension. Their histological descriptions may be summarized as follows: The earliest lesion is a "non-fatty" nodule of the glomerular zone involving to some extent the fascicular zone. This change may be found in other conditions, but is more marked in chronic interstitial nephritis. Another early appearance is a "fatty" nodule well limited and isolated in the midst of normal cells and seen especially in the fascicular zone. The latter may sometimes be very numerous and not sharply limited. The final stage is a *hyperplasia adenomatosa totale* with definite increase in the weight of the gland. A true macroscopic adenoma of the adrenal they state to be of no significance, unless associated with diffuse hyperplasia. It differs from the so-called adenoma of nephritis in that the latter is a more marked or accentuated local manifestation of a diffuse process, while the ordinary macroscopic adenoma develops in an otherwise normal gland.

[6] Aubertin, C., and Ambard, L., Lésions des capsules surrénales dans les néphrites avec hypertension, *Bull. et mem. d. l. soc. med. d. hôp. d. Paris*, 1904, xxi, 175.

[7] Vaquez and Aubertin, C., Sur l'hyperplasie surrénale des néphrites hypertensives, *ibid.*, 1905, xxii, 705.

It must be here stated that these writers have never limited these lesions to the adrenal of chronic interstitial nephritis but have said, that if individuals of very advanced age are eliminated, the lesions are found in nineteen of twenty cases of the interstitial type of nephritis and but once in twenty cases of the parenchymatous type, or in other diseases than interstitial nephritis. They do insist, however, that these lesions are always accompanied by hypertension except in those cases of chronic nephritis running a very rapid course.

The material of the present study consists of 163 adrenals which have been divided into groups according to the ages of the individual and the presence or absence of chronic renal and vascular lesions. They were selected from a group of 1,200 autopsies and include all sections of the adrenal which pass through the center of the entire gland and are well preserved and well stained. They are therefore representative of the lesions occurring in the average run of autopsy material. In no case, however, have we notes of the blood pressure during life. For this reason the study of material of this kind is not comparable, strictly speaking, to the studies of the French investigators as the latter, for the most part, used only material from individuals who during life showed definite hypertension. The purely anatomical studies here presented are, however, of value as control observations and form a basis for a detailed study, now in progress, of the adrenal from individuals exhibiting hypertension clinically.

Group I. Normal Glands.—This group includes twelve glands from individuals between forty and fifty years of age (average forty-four years) who met sudden death from some form of violence. They are taken as normal controls.

Group II. Glands from Individuals under Thirty Years of Age Dying of Infectious Diseases.—This group includes forty-seven glands of which fourteen are from infants and sixteen from children. They were taken as controls of the acute degenerative or other lesions which might be caused by infection. The diseases represented are diphtheria, typhoid fever, tuberculosis and noma, acute infections of the respiratory system, and diseases of the gastro-intestinal tract, middle ear, and uterus. In only eight ex-

amples could departures from the normal structure be found. These occurred in three glands of general tuberculosis, in two of typhoid fever, and one gland each of diphtheria, pelvic abscess and septic endometritis. All were in individuals over twenty years of age. In one gland of the tuberculosis group, a diffuse hyperplasia of the fascicular zone with the characteristic spongy appearance, and in another a very definite nodular hyperplasia of the glomerular zone occurred; in two excessive pigmentation and in all a hyperplasia of the medulla existed. In one also a slight diffuse increase of connective tissue was present and in another some round cell infiltration. In the case of acute septic endometritis a very definite hyperplasia of the medulla with round cell infiltration existed. In one gland from a case of typhoid fever were noted focal necroses with extensive leucocytic infiltration and in the other multiple nodular hyperplasia of the glomerular and fascicular zones and diffuse hyperplasia of the medulla. In the glands from cases of diphtheria and pelvic abscess nodular adenomata were also present with more or less diffuse "fatty" changes. In connection with the changes in the adrenals of tuberculous individuals here described it is noteworthy that Aubertin and Clunet[8] have described hyperplasia of the medulla in five cases of tuberculosis.

Group III. Glands from Individuals over Thirty Years of Age, Free of Chronic Vascular and Renal Disease.—This group includes nineteen glands from individuals between thirty-one and fifty-seven years of age (average forty-four years). They are to be regarded as controls for the glands derived from individuals of the arteriosclerotic period. Eight showed no adenomatous changes, while in eleven were found the changes described by Vaquez and Aubertin. The hyperplasia was nodular in eight, diffuse in three and combined in two; in three of the nodular variety the lesion was of the "fatty" type. Increase of connective tissue was found in three glands from cases of syphilis, tuberculosis and cancer, respectively, with, in the first, considerable round cell infiltration. Excessive pigmentation was present in two glands of chronic mitral endocarditis. Hyperplasia of the medulla occurred in two instances

[8] Aubertin, C., et Clunet, J., Hypertrophie cardiaque et hyperplaise médullaire des surrénales, *C. r. Soc. de Biol.*, 1907, lxiii, 595.

in association with syphilis and chronic mitral disease, an association which has also been noted by Aubertin and Clunet.

Group IV. Glands from Individuals over Thirty Years of Age, with Contracted Kidney, Arteriosclerosis and Heart Hypertrophy.—This group has been very carefully selected and comprises twenty-four glands from individuals who suffered from typical chronic nephritis of the interstitial type (contracted kidney) associated with well-marked general arteriosclerosis, involving the aorta or its larger branches, and with hypertrophy of the left ventricle (in four instances both ventricles) of the heart. That is, it represents the anatomical material corresponding to the typical clinical features of chronic vascular and renal disease with which hypertension is usually associated. The ages varied from thirty-eight to eighty-seven years (average, fifty-nine years).

Only one of these glands could be considered absolutely normal. Sixteen showed changes which must be considered as the result of the effect on the gland of the arteriosclerotic changes in its own vessels. These changes are similar to those found in the kidney, pancreas and other organs when their vessels are altered by arteriosclerosis. The vessels of the medulla and capsule show fibrous and hyaline thickening with diminution of lumen, the capsule is thickened and both cortex and medulla show various grades of connective tissue increase and round cell infiltration.

In all instances, in addition to these productive changes, certain lesions described by the French writers were present to some degree, and most frequently the diffuse hyperplasia combined with simple nodules of either the "fatty" or "non-fatty" type.

Similar hyperplastic lesions were found in the seven glands in which evident fibrous and local vascular changes were absent, though in three of them, it should be noted, round cell infiltration was a prominent condition.

In six glands of this group a definite hyperplasia of the medulla was apparent, and in two pigmentation was very marked; in almost all the glands it was greater than normal.

The relation of the adenomatous changes to the local lesions due to arteriosclerosis, in the sixteen glands first described, might be explained as compensatory to gland injury and analogous to the

"multiple nodular hyperplasia" seen in the liver in cirrhosis and in repair of acute yellow atrophy or after other destructive lesions. This explanation cannot on the other hand be applied to the simple hyperplasia observed in the seven glands without connective tissue proliferation. The hyperplasia however is no more frequent in these glands than in Groups II and III in which chronic vascular and renal lesions do not come into question.

Group V. Arteriosclerosis Associated with Chronic Nephritis of the Parenchymatous Type.—This group includes thirteen glands from individuals who showed arteriosclerosis of the same grade as the previous group, but in whom chronic parenchymatous nephritis instead of the interstitial type existed. This material was very carefully selected in order to exclude the latter and to include examples of general vascular lesions of the same degree as in the latter disease. The ages varied from thirty-eight to eighty years (average, fifty-seven years).

Of these glands two were normal, five showed vascular and connective tissue lesions of the character seen in Group IV, associated in three with hyperplasia, and six showed hyperplasia only.

The conditions therefore are not essentially different from those of the previous group and do not support the view of the greater importance of chronic interstitial nephritis as productive of hyperplasia of the adrenal.

Group VI. Arteriosclerosis without Chronic Nephritis.—This group comprises sixteen glands obtained from individuals with well-marked arteriosclerosis of the aorta and larger vessels but without chronic nephritis of any type. The fatalities include various infections and the chronic diseases common to the latter half of life. The ages vary from thirty-five to seventy-four years (average, fifty years).

Of the sixteen glands, ten showed the vascular and connective tissue changes previously described as characteristic of the arteriosclerotic gland. In all but three of these, adenomatous lesions also exist. Four exhibited hyperplasia only, and two were normal.

These observations compared with those made of Groups IV and V indicate that the constant factor in producing the hyperplasia is arteriosclerosis; and hyperplasia occurs indifferently in chronic

nephritis of the parenchymatous type, of the interstitial type, and in absence of chronic nephritis of all types.

Group VII. Chronic Nephritis without Arteriosclerosis.—This group includes twelve glands obtained from individuals without arteriosclerosis but who had definite chronic nephritis, which in ten was of the parenchymatous and in two of the interstitial type. Incidentally this group bears on the question of the frequency with which chronic interstitial nephritis occurs in the absence of arterisclerosis. The ages ranged from twenty-three to fifty years (average, thirty-six years).

Five of the glands were normal. Two, from individuals with pneumonia and gangrene of the leg, respectively, showed diffuse hyperplasia with discrete non-fatty nodules; and four showed nodular adenomata only. The remaining gland from an individual with tertiary syphilis presented very extensive round cell infiltration of the medulla, but no hyperplasia. Extensive round cell infiltration was present also in the gland from an individual with gangrene of the leg; and slight infiltration in one of chronic mitral disease. In no instance was an increase of connective tissue present. Of the two cases of interstitial nephritis one gland showed nodular hyperplasia and the other none.

These findings are in accord with those previously presented as far as the influence of chronic nephritis of the interstitial type is concerned and indicate, as do the findings in Groups II and III, the relative frequency of hyperplasia of the adrenal in the absence of arteriosclerosis.

Group VIII. Does Hyperplasia Occur in Chronic Destructive Lesions of the Adrenal?—In another section (Group IV) the statement has been made that the adenomatous hyperplasia of the adrenal, in chronic productive lesions of the gland, might be in part a compensatory process analogous to the nodular hyperplasia occurring in the liver of cirrhosis. An attempt has been made to determine whether such a hyperplasia occurs in the persisting tissue of adrenals which are the seat of extensive chronic destructive processes. Two glands invaded by carcinoma, two with amyloid and ten with tuberculosis have been studied. In two of these, cancer and amyloid respectively, a nodular hyperplasia of the "non-fatty" type was demonstrable in the glomerular and fascicular zones, while

in four of the tuberculous organs areas were found which could be interpreted only as the result of hyperplasia. These masses do not show the normal architecture of the adrenal, but are atypical and consist of very large pale vacuolated cells usually containing considerable pigment. The masses are located, frequently, at a distance from the tuberculous lesions and encased in connective tissue. In one gland in which considerable portions of the organ remained intact a diffuse hyperplasia was present.

These findings support the theory of a compensatory nodular hyperplasia of the adrenal, and suggest a possible explanation of the relatively constant hyperplasia of the arteriosclerotic gland.

Group IX. Macroscopic Adenoma of the Adrenal and Kidney (Hypernephroma).—Although Vaquez and Aubertin state that macroscopic adenomata are of no importance in the production of hypertension or in the relation of the adrenal to chronic nephritis, I have grouped these cases in order to present the subject in complete form.

Three hypernephromata of the kidney and three adenomata of the adrenal were available for study. One of the latter was from an individual of thirty-eight years of age who was free from renal and cardio-vascular lesions. The other five were all from individuals between fifty and sixty years of age. Chronic nephritis of the parenchymatous type was present in two and of the interstitial type in two individuals; and the kidney was normal in one individual. In three of the cases a well-marked general arteriosclerosis and in two slight lesions of the aorta and coronary arteries existed. Heart hypertrophy was absent in all.

Macroscopic adrenal adenomata would therefore appear to have no definite relation to chronic interstitial nephritis; whether they bear any relation to arteriosclerosis cannot be concluded from the small number of examples in my studies.

SUMMARY.

Vaquez and Aubertin advance three theories in explanation of the adrenal hyperplasia; first, that it may not be the cause of hypertension but "an antitoxic hyperplasia" caused by the retained products of metabolism which may be responsible also for the hypertension; second, that it may be the cause of hypertension but

secondary to the renal lesion; third, that it may be the cause of hypertension but may antedate the renal lesion or be entirely independent of it. They, as well as other French writers, insist that this hyperplasia is almost constantly associated with chronic nephritis of the interstitial type and it is seldom found with the parenchymatous type of nephritis, or with other lesions.

Hyperplasia of the adrenal, as far as my material enables one to judge, does not occur during the first and second decades. In the third decade it is relatively frequent in the absence of chronic arterial and renal disease but reaches the maximum in association with such disease after the fourth decade. It is an almost constant lesion in arteriosclerosis associated with chronic interstitial nephritis and left-sided heart hypertrophy, but occurs with almost equal frequency in arteriosclerosis with chronic nephritis of the parenchymatous type. It is a relatively frequent lesion of arteriosclerosis without chronic nephritis and of the latter without arteriosclerosis also. As the result of this analysis one is led to the view that while hyperplasia of the adrenal is a very frequent concomitant of chronic renal and arterial disease it is not exclusively a feature of either type of nephritis or yet of chronic vascular disease; but it probably represents the effect of some factor operating in that period of life in which chronic renal and arterial affections are most frequent.

Worthy of special emphasis is the observation that the characteristic lesion of an adrenal, the seat of local arteriosclerosis, is of the type of the chronic productive inflammation seen in arteriosclerosis of the pancreas and kidney; that is, thickening of the vessels, increase of connective tissue and round cell infiltration. Associated with these changes is a hyperplasia which is very constant, and which may be, in part, of the nature of a compensatory hyperplasia similar to that seen in the liver of cirrhosis and acute yellow atrophy. A hyperplasia of this type, as has been shown, may occur in destructive lesions of the gland. This, however, does not explain hyperplasia in the absence of local vascular changes which fact is, possibly, as suggested by Landau, evidence, not of a correlation between kidney and adrenal, but of a vicarious hypertrophy depending upon lesions of some other organ of the body than the kidney, possibly some other ductless gland, affected by arteriosclerosis or other disease.

A STUDY OF EXPERIMENTAL REDUCTION OF KIDNEY TISSUE WITH SPECIAL REFERENCE TO THE CHANGES IN THAT REMAINING.[1]

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The following experiments in reduction of kidney tissue in dogs were undertaken in order that one of the writers might study the influence of such reduction on nitrogenous metabolism[2] and also for the purpose of determining the effect of this procedure on the remaining kidney tissue and upon the general condition of the animal.

The scanty literature of the subject may be briefly reviewed as follows:

The most extensive series of kidney reduction is that of Bradford.[3] The operative procedure of this investigator was to remove from the middle of the kidney, entering the pelvis, a wedge-shaped mass of kidney substance. This mass was equal to one quarter to one half of the entire kidney. After a variable period of time the entire opposite kidney was removed. In some instances a wedge was taken from each kidney and later the remaining tissue still further reduced. Of a total of thirty-three dogs, four died after the first operation and six at or shortly after the second operation. Of the twenty-three surviving the second operation, eight lived a considerable period of time and were killed in good health. The effect on the kidney of the first operation was usually atrophy of the organ. This occurred also if both kidneys were operated upon. After the second operation no serious disturbance of health occurred but a variable amount of wasting, usually transitory, was the rule.

If two thirds of the kidney was removed death usually followed while the loss of three quarters rendered survival impossible, death occurring usually in one to six weeks, from asthenia and great wasting.

Bradford found that two grams of kidney tissue per kilo of body weight was sufficient for maintenance of life (normal ratio 6.7 grams). Tuffier[4] gives

[1] Aided by a grant from the Rockefeller Institute for Medical Research. Received for publication July 15, 1908.

[2] Pearce, R. M., The Influence of the Reduction of Kidney Substance on Nitrogenous Metabolism, *Jour. of Exper. Med.*, 1908, x, 632.

[3] Bradford, J. R., The Results Following Partial Nephrectomy and the Influence of the Kidney on Metabolism, *Jour. of Physiol.*, 1899, xxiii, 415.

[4] Tuffier, T., Etudes expérimentales sur la chirurgie du rein, Paris, 1899 (quoted by Bradford).

1.5 grams, Paoli[5] states that one half a kidney is necessary. The latter found the operation to be followed frequently by hypertrophy of the glandular epithelium, dilatation of the vessel and a new formation of glomeruli and tubules. Tuffier's operation differed from Bradford in that he removed one entire kidney first and later a portion of the other. As a result of ten experiments he found no permanent disturbance of the urine and no effect on the development of growing animals.

Von Haberer's[6] experiments on three goats and 38 dogs are most unsatisfactory because many of his animals died of pneumonia. He practiced very extensive resections within very short periods of time and showed that it is possible for a dog to survive the removal of one kidney and one third of the other at a single operation or one and a half at two operations within a week. The mortality was very high.

Passler and Heineke[7] in their work on experimental heart hypertrophy, practiced extensive kidney reduction. They followed Bradford's method, allowing an interval of four weeks between the first and second operation; in 5 dogs cachexia and death followed. In eighteen surviving the second operation, at intervals varying from a few days to eleven months, a third, fourth, fifth or sixth operation was done, at each a small portion of kidney substance being removed. All finally died, from operations, accident or cachexia. As their interest was chiefly in heart hypertrophy, the details of the effect of reduction on the kidney tissue remaining are not given.

Bainbridge and Beddard,[8] working with cats according to Bradford's method, reduced the kidney substance three quarters. This caused loss of appetite, wasting and death in a few days or weeks. Descriptions of the effect on the remaining kidney tissue are not given.

METHODS.

As our operative procedure differed from that of the other investigators it is here given in detail. If only one kidney was operated upon, it was exposed through a so-called "gridiron" incision in the lumbar region by separating the muscle fibers and not cutting them. If both kidneys were exposed at one operation, this was done transperitoneally through a median abdominal incision. In the first few experiments the pedicle of the kidney was compressed by an assistant while the upper pole was excised

[5] Paoli, Della resezione del rene *Cent. f. Chirurgie*, 1892, xix, 78.

[6] V. Haberer, H., Experimentelle Untersuchungen über Nierenreduktion und Funktion des restierenden Parenchyms, *Mitteilungen a. d. Grenzgebieten d. Med. u. Chir.*, 1907, xvii, 57.

[7] Passler and Heineke, Versuche zur Pathologie des Morbus Brightii, *Verhandl. d. Deutsch. path. Gesellsch.*, 1905, ix, 99.

[8] Bainbridge, F. A., and Beddard, A. P., The Relation of the Kidney to Metabolism, *Proc. of the Royal Soc.*, B, 1907, lxxix, 75.

and then the bleeding was controlled by matress sutures of catgut and silk passing through the entire kidney substance and the upper portion of the pelvis. Later it was found easier to place catgut sutures first as shown in Figs. 1 and 2, tie them and excise the upper pole, the operation usually being bloodless, and possible without an assistant.

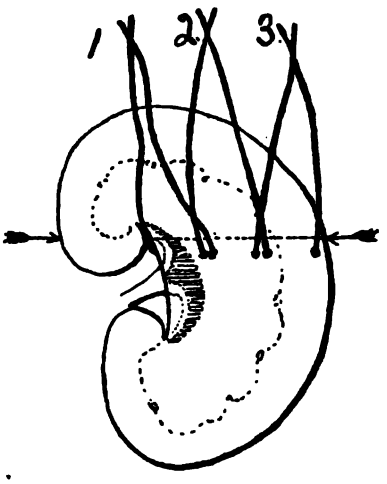

FIG. 1.

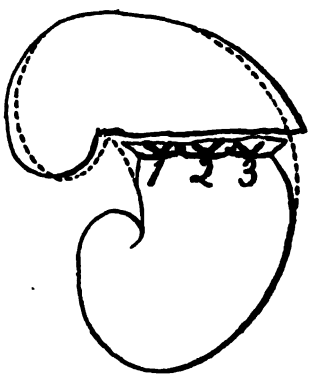

FIG. 2.

FIG. 1. Technique of resection of upper pole of the kidney.

By means of a straight needle with a dull point, three interlacing mattress catgut (No. 2 Cumal) sutures (1, 2 and 3) are first passed through the kidney in a line which approximately divides the kidney in half (see illustration). Care must be taken to avoid injuring the ureter and for this reason the line of sutures is taken a little obliquely rather than straight across the kidney. These sutures are then tied, thus completely shutting off the blood supply to the upper pole. The upper pole is cut off from 2 to 4 mm. beyond the sutures as indicated by the arrows.

FIG. 2. Immediate result of resection of upper pole of the kidney.

The dotted line indicates the original outline of the upper pole. After excising it, the base of the upper pole flares apart and the segment is thus lower and wider than before the operation. The sutures 1, 2 and 3 have been tied, thus constricting the upper portion of the remaining kidney tissue and by making the capsule more tense the part of the kidney, left behind, is a trifle smaller than before the operation.

This operative procedure we have considered preferable to that of Bradford and other investigators because there is a minimum disturbance of the blood supply of the kidney tissue remaining.

The infarcted tissue, which results from the injury, and must be absorbed, lies at one pole of an otherwise intact kidney while in the experiments of Bradford and others, it is centrally located and thus possibly disturbs the nutrition of the entire organ. Our method furthermore permits of a more accurate estimation of the amount of kidney reduction in that the degree of reduction is represented by the part excised plus that outside of and that in the immediate neighborhood of the sutures. Histological examinations of kidneys from animals dying shortly after operation have shown that the zone of necrosis rarely extends more than one half centimeter from the sutures into the remaining kidney tissue. By the other method, after the removal of a wedge-shaped piece of tissue from the midst of the organ, the sutures necessary to approximate the parts remaining and to control bleeding of necessity destroy a mass of kidney tissue which in proportion to that excised must be much greater than that destroyed in removing one pole. By our method a portion of the pelvis is necessarily removed and there is some danger of interfering with the flow of urine into the ureter. By proper care we have found that this can be avoided.

Except for the animals used for the metabolism studies above mentioned, the dogs were fed on dog biscuits, and did not have any excercise other than that possible in fairly roomy cages. The general condition of the animals was determined by observations in regard to appetite and weight.

All material obtained at operation or autopsy was preserved in ten per cent. formalin. No subdivisions of this material were made but large sections were obtained which represented the entire mass. By this method it was possible to study in one section, cortex, pyramid, pelvis and reparative or other changes in the field of operation. By superimposing these large sections it was possible to compare directly the remaining kidney tissue with that removed at operation and also to make tracings for comparison and illustrations.

Our experiments are sixteen in number, of which two (one death from anæsthesia and another from hemorrhage shortly after operation) are excluded. The remaining fourteen experiments are divided into four groups.

Group I. Removal of Upper Pole of One Kidney.

No. 1 (Dog 40).

Operative Procedure.—Jan. 23, '08. Under ether narcosis, the upper pole of the left kidney was removed as shown in Figs. 1 and 2. The length of the left kidney was 5.6 cm. and the portion remaining outside of the sutures was 2.7 cm. At the same time the right kidney was exposed and measured.

Termination.—Killed on the 22nd day.

General Effect.—Post-operative history was uneventful. There was a loss of weight from 11,020 to 9,710 grams.

Effect on Remaining Kidney Tissue.—Slight but definite atrophy. Healing is perfect, the renal tissue, compressed by the sutures, is replaced by fibrous tissue except for a small area of infarcted tissue corresponding to vessels showing obliterated endarteritis. In the fibrous tissue adjacent to the pelvis there is a small area of new bone formation. Otherwise the renal tissue is normal. The opposite kidney is apparently unaffected by the operation.

No. 2 (Dog 41).

Operative Procedure.—Jan. 23, '08. Under ether narcosis, the upper pole of the left kidney was removed. The length of the left kidney was 6 cm. and the portion remaining outside of the sutures was 3 cm. At the same time the right kidney was exposed and measured.

Termination.—Killed on the 35th day.

General Effect.—Post-operative history was uneventful. There was a loss of weight from 13,610 to 10,960 grams. Four weeks after the operation the urine contained a considerable amount of coagulable protein with numerous finely granular and fatty casts, pus and epithelial cells (urine was not examined before operation).

Effect on Remaining Kidney Tissue.—Very slight if any atrophy. Healing is perfect except for a few areas of necrotic tissue near the pelvis. In the scar there is an area of calcified tubules. Pelvic epithelium is greatly thickened and penetrates the underlying scar tissue, forming alveolar masses of epithelial cells. Otherwise the renal tissue is normal. Opposite kidney is apparently unaffected by the operation.

No. 3 (Dog 31).

Operative Procedure.—Dec. 31, '07. Under ether narcosis, the upper pole of the right kidney was removed. The length of the right kidney was 6.2 cm. and the portion remaining outside of the sutures was 3.5 cm. At the same time the right kidney was measured.

Termination.—Killed on the 48th day.

General Effect.—Post-operative history was uneventful. There was a loss of weight from 10,620 to 9,650 grams. Urine, on the

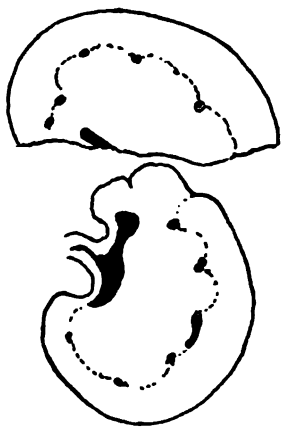

FIG. 3. Tracings of sagittal sections of portions of a kidney, one portion removed at operation and the other at autopsy on the thirty-fifth day.

The upper pole was removed at operation as indicated in Figs. 1 and 2 and shows the characteristic flaring apart of the segment removed. The lower portion was removed at autopsy and has apparently atrophied but, by comparing it with a similar tracing of the opposite kidney and even from the measurements taken at the close of the operation (see Figs. 2), there is very little if any atrophy except in the tissue destroyed by the sutures and replaced by scar tissue. For description of microscopical findings see Experiment No. 2.

fourth day, was normal but at the time of autopsy contained coaguable protein and pus cells, but no casts.

Effect on Remaining Kidney Tissue.—Very slight if any atrophy exists. Healing is perfect. There is dilatation of the pelvis of the kidney with a chronic pyelitis and thickening of the epithelium, and extensive bone formation immediately beneath this epithelium. Calcified tubules are present in the scar tissue. Otherwise the renal

tissue shows atrophy of glomeruli with fibrous thickening of Bowman's capsule and extensive round cell infiltration of both cortex and pyramid. The tubules in places are dilated and in other places compressed. The portion of kidney removed at operation and the opposite kidney shows a spontaneous chronic nephritis but less marked than that just described.

Group II. Removal of Upper Pole of Each Kidney at One Operation.

No. 4 (Dog 65).

Operative Procedure.—March 7, '08. Under ether narcosis. the upper pole of each kidney was removed. The length of the right kidney was 4.5 cm. and the portion remaining outside of the sutures was 2.3 cm. The length of the left kidney was 4.4 cm. and the portion remaining outside of the sutures was 2.6 cm.

Termination.—Died on 30th day.

General Effect.—Post-operative history was uneventful until the last week, when the animal refused food, lost weight rapidly and died on the 30th day.

Effect on Remaining Kidney Tissue.—No change in the size of the portion of the right kidney remaining. Healing was perfect except for a small area of necrosis along the line of suture, thickening of epithelium of pelvis and isolated masses of proliferating epithelium in the scar tissue. Otherwise the remaining renal tissue is normal.

The pelvis of the left kidney is filled with calculi, one occluding the lumen of the ureter. Associated with this there is an ulcerative pyelitis with abscesses involving the pelvic fat and lower portion of the pyramid. Where pelvic epithelium is intact it shows hyperplasia. There is no definite atrophy of cortex and pyramid.

No. 5 (Dog 66).

Operative Procedure.—March 7, '08. Under ether narcosis, the upper pole of each kidney was removed. The length of the right kidney was 4.9 cm. and the portion remaining outside of the sutures was 3 cm. The length of the left kidney was 4.8 cm. and the portion remaining outside of the sutures was 2.6 cm.

Termination.—Killed on the 55th day.

General Effect.—Post-operative history was uneventful. There was a loss of weight from 4,620 to 4,340 grams.

Effect on Remaining Kidney Tissue.—There is no change in the size of the portion of the right kidney remaining. Healing is perfect. There is moderate thickening of the epithelium of the pelvis. Renal tissue is normal.

There is no change in the size of the portion of the left kidney remaining, and it is similar to the right kidney.

No. 6 (Dog 1).

Operative Procedure.—May 23, '07. Under ether narcosis the upper pole of each kidney was removed. The length of each kidney was 6 cm. and the portion remaining outside of the sutures was 3 cm. Weight of portion of right kidney removed was 10 grams and of left 11 grams.

Termination.—Died on 164th day, from general peritonitis, the fifth day after the removal of the remaining portion of the left kidney when the peritoneal cavity was opened and soiled with infected urine.

General Effect.—Post-operative history was uneventful. The dog was in the country during the summer and became pregnant. The weight at the time of the first operation was 13,180 grams; at the second operation (pregnant) 16,010 grams and after death with the uterus empty (aborted just before death) it was 12,110 grams.

Effect on Remaining Kidney Tissue.—Left kidney weighed 18 grams, length 4.8 cm., an increase of 1.8 cm. There was a small calculus in the pelvis about a silk suture. Healing is perfect, kidney has assumed its normal shape and except for the presence of a silk suture it would be difficult to distinguish it from a normal kidney. Microscopically there is evidence of pyelitis.

Right kidney weighed 28 grams, length 5.1 cm., an increase of 2.1 cm. above the portion remaining beyond the sutures at operation. Healing is perfect, microscopically renal tissue is normal. There is bone formation beneath the epithelium of the pelvis.

Group III. Removal of One Kidney and Upper Pole of the Other at One Operation.

No. 7 (Dog 35).

Operative Procedure.—Jan. 23, '08. Under ether narcosis the right kidney was removed and the upper pole of the left. The length of the left kidney was 6 cm. and the portion remaining outside of the sutures was 3.2 cm.

Termination.—Killed on the 6th day (dying).

General Effect.—There was persistent vomiting after operation with gradual loss of strength, and failure to eat; a small amount of urine was passed daily.

Effect on Remaining Kidney Tissue.—There is no change in the size of the portion of the kidney remaining. Extensive areas of infarction about the sutures are present with widespread calcification of the tubules. Infarcted area is 1.5 cm. wide, the line of sutures being in the middle of this area. Abscess formation present about sutures.

No. 8 (Dog 69).

Operative Procedure.—April 6, '08. Under ether narcosis the right kidney was removed and the upper pole of the left. The length of the left kidney was 5.7 cm. and the portion remaining outside of the sutures was 3.5 cm.

Termination.—Killed on the 7th day (dying). Cause of death not apparent.

General Effect.—Refused food and vomited persistently until killed on the 7th day. There was a loss of weight from 9,710 to 9,620 grams. Urine was voided after operation but was not examined.

Effect on Remaining Kidney Tissue.—There is no change in the size of the portion of the kidney tissue remaining. The area of infarction and hemorrhage is 1.7 cm. broad, the line of sutures being in the middle of this area. Very slight leucocytic infiltration about the sutures is present. There is marked proliferation of the pelvic epithelium.

No. 9 (Dog 24).

Operative Procedure.—Dec. 9, '07. Under ether narcosis the

right kidney was removed and the upper pole of the left. The length of the left kidney was 6.3 cm. and the portion remaining outside of the sutures was 3.5 cm.

Termination.—Died on the 10th day.

General Effect.—Ate poorly, lost 500 grams in the first five days after the operation, hematuria.

Effect on Remaining Kidney Tissue.—There is no change in the size of the remaining portion of the left kidney. Infarcated area varies from 1 to 1.3 cm., the sutures being nearly at the junction of the normal and necrotic tissue. Otherwise the kidney is normal.

No. 10 (Dog 21).

Operative Procedure.—Dec. 3, '07. Under ether narcosis the right kidney was removed and the upper pole of the left. The length of the left was 5.2 cm. and the portion remaining outside of the sutures was less than half the original length of the kidney.

Termination.—Killed on the 35th day.

General Effect.—Post-operative history was uneventful. There was a loss of weight from 8,410 to 8,050 grams. Slight albuminuria and pyuria.

Effect of Remaining Kidney Tissue.—There is no change in the size of the portion of the kidney tissue remaining. Healing is perfect; a few calcified tubules are present in the scar tissue. Otherwise kidney tissue is normal.

No. 11 (Dog 72).

Operative Procedure.—March 21, '08. Under ether narcosis the right kidney was removed and the upper pole of the left. The length of the left kidney was 6 cm. and the portion outside of the sutures was 3.2 cm.

Termination.—Killed on the 41st day.

General Effect.—Post-operative history was uneventful. There was a loss of weight from 15,060 to 12,340 grams. Urine obtained from bladder at autopsy was normal.

Effect on Remaining Kidney Tissue.—There is no change in the size of the portion of the kidney tissue remaining. Healing is perfect; there are a few calcified tubules in the scar tissue. As compared with portion excised at operation and the opposite kidney

there is no change in the renal tissue. Each show a chronic process characterized by round cell infiltration.

No. 12 (Dog 71).

Operative Procedure.—March 21, '08. Under ether narcosis, the right kidney was removed and the upper pole of the left. The length of the left kidney was 5.4 cm. and the portion remaining outside of the sutures was 3 cm.

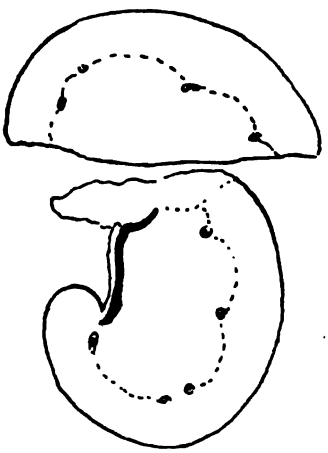

FIG. 4. Tracings of sagittal sections of portions of a kidney, one portion removed at operation and the other at autopsy on the forty-first day.

For description of gross appearance see Fig. 3 and for description of microscopical findings see Experiment No. 11.

Termination.—Killed on the 57th day.

General Effect.—Post-operative history was uneventful. There was a loss of weight from 8,010 to 6,060 grams.

Effect of Remaining Kidney Tissue.—There is no change in the size of the portion of the kidney tissue remaining. Healing is perfect, there are a few calcified tubules in the scar tissue, as in No. 11.

Group IV. Reduction of Kidney Tissue by Multiple Operations.

No. 13 (Dog 4).

Operative Procedure.—Oct. 24, '08. Under ether narcosis the upper pole (over ½ approximately ⅔) of the right kidney was removed. Nov. 8, '08. The upper pole of the left was removed.

The length of the left kidney was 6.5 cm. and the portion remaining outside of the sutures was 3 cm. Dec. 11, '08. The remainder of the left kidney was removed.

Termination.—Died four hours after the last operation from œdema of the lungs.

General Effect.—Post-operative history was uneventful. There was a loss of weight from 13,826 to 12,250 grams and there were periodic albuminuria and pus cells.

Effect on Remaining Kidney Tissue.—There is slight if any atrophy of remaining portion of right kidney. Healing is perfect. A cheesy material containing calculi is present in the pelvis; bone formation in scar tissue beneath the pelvic epithelium; marked hyperplasia of pelvic epithelium. Kidney tissue is normal except for areas of round cell infiltration, also present in portion excised at first operation.

No change in the size of the remaining portion of the left kidney. Healing is perfect with extensive bone formation beneath the pelvic epithelium. In the pelvic fat there is a small abscess about one of the sutures. Otherwise tissue is as in the opposite kidney.

No. 14 (Dog 13).

Operative Procedure.—Nov. 2, '07. Under ether narcosis the upper pole of the left kidney (weight 14.5 grams) was removed. The length of the left kidney was 6.2 cm. and the portion remaining outside of the sutures was 2.7 cm. Nov. 13, '07. The right kidney was removed (weight 42 grams). Dec. 9, '07. A segment 1 cm. thick was removed (weight 8 grams) from the lower pole of the remaining portion of the left kidney.

Termination.—Died 11 days after the last operation.

General Effect.—Good recovery from each operation except the last. More or less vomiting after the last operation, albuminuria one half to two per cent., abundant casts; voided urine until death and averaged 180 c.c. for the last three days. There was a loss of weight from 15,840 to 10,000 grams.

Effect on Remaining Kidney Tissue.—The portion of the left kidney found at autopsy showed distinct atrophy and weighed 11 grams. Based on the weight of the normal right kidney this repre-

sents 14 per cent. of the original kidney tissue. Healing from the first operation is perfect and site of second operation is occupied by a mass of necrotic tissue extending into the pelvis and surrounded by purulent fluid. Microscopically there is evidence of an acute ulcerative pyelitis with slight pyelonephritis and extensive cast formation in the tubules. Otherwise kidney tissue is normal.

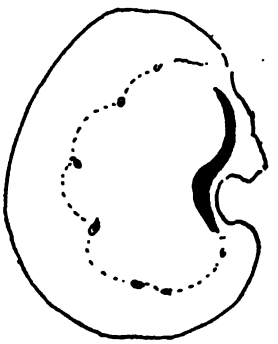

FIG. 5.

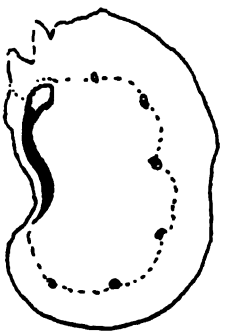

FIG. 6.

FIG. 5. Tracing of sagittal section of portion of the right kidney remaining, 164 days after removal of the upper pole.

The upper pole was removed as indicated in Figs. 1 and 2.

The portion remaining shows distinct hypertrophy and the thickening of the cortex is most evident near the field of the operation. See Experiment No. 6.

FIG. 6. Tracing of sagittal section of the portion of the left kidney remaining, 159 days after removal of the upper pole.

The upper pole was removed as indicated in Figs. 1 and 2.

This is from the same dog as the kidney shown in Fig. 5, the hypertrophy is not as marked as in the other kidney but the thickening of the cortex near the field of operation is more marked and the kidney has almost resumed its normal contour. A calculus about a silk suture is present in the upper portion of the pelvis.

GENERAL SUMMARY.

The immediate effect of the operation on the portion of the kidney remaining is an infarcation of the tissue compressed by the sutures. This area of necrosis extends but a short distance into the adjacent kidney tissue. The infarcated tissue gradually becomes replaced by fibrous tissue and in three to four weeks' time the necrotic tissue entirely disappears. The amount of fibrous tissue in time becomes so slight and the healing so perfect that it is difficult to detect the site of the operation. The renal elements sometimes persist in the infarcated area and the glomeruli ap-

parently are more resistant than the tubules. The tubules in the infarcated area sometimes become calcified and bone formation beneath the epithelium of the pelvis is of very frequent occurrence. The pelvic epithelium usually shows marked proliferation and may invade the field of operation in alveolar masses. Calculi may form in the pelvis of the kidney. Sutures penetrating the pelvis as well as the necrotic tissue resulting from the compression of the sutures probably furnish the nuclei for the calculi.

Removal of approximately half of one kidney did not alter either the remaining portion of that kidney or the size of the opposite kidney in two animals of this series. These experiments were terminated on the thirty-fourth and forty-seventh days. In a similar experiment of twenty-one days' duration there was slight but definite atrophy of the remaining tissue of the kidney operated upon.

Removal of approximately half of each kidney at one operation did not alter the remaining kidney tissue in two animals of the second group. The longest period of observtaion was fifty-four days. On the other hand in a similar experiment where 164 days elapsed, the remaining portions of each kidney had increased markedly in size.

Removal of one kidney and approximately half of the other did not alter the remaining kidney tissue in six animals in which from five to fifty-six days elapsed before termination of the experiment.

In six experiments in which one kidney was removed and approximately half of the other, three of the animals died as a result of the operation on the sixth, seventh and tenth days. The probable cause of death was renal insufficiency; the animals refused food, vomited persistently and lost strength and weight. The other three animals recovered and were killed at periods varying from five to eight weeks. The reduction of the kidney tissue to one quarter of its original amount at one operation was attained with danger but was not necessarily fatal.

In every experiment there was a loss of weight varying from four to twenty-four per cent. To what extent this loss in weight is due to the reduction of kidney substance and to what extent it is due to diet and confinement it is impossible to say, as we made no control experiments to elucidate these points.

ON THE OCCURRENCE OF CATALASE IN HUMAN TISSUES AND ITS VARIATIONS IN DISEASES.[1]

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Catalase is a ferment which occurs in all vegetable and animal tissues, and is characterized by its power of decomposing hydrogen peroxide with the formation of water and the evolution of oxygen. Schoenbein in his studies on hydrogen peroxide, found that it was decomposed by extracts of animal and plant tissues obtained from the most diverse sources, and he considered that all ferments possessed the property of decomposing hydrogen peroxide, which besides was able to effect their specific activity. His experiments showed not only that some enzymes did not have the power of splitting hydrogen peroxide, but that by the addition of weak acids and other inhibitors, enzymes could be made to lose their power of decomposing hydrogen peroxide without losing their specific action, for example, by heating emulsin or by adding weak acids to it, its action on hydrogen peroxide decreased more rapidly than its action on amygdalin. This was the first indication of the specific nature of the enzyme which catalytically decomposes hydrogen peroxide, namely, catalase.

The property tissue extracts have of producing a blue coloration with tincture of guaiac in the presence of hydrogen peroxide was also thought by Schoenbein to be brought about by the various soluble ferments. He obtained both of the above reactions—the guaiac blueing test in the presence of hydrogen peroxide, and the

[1] Received for publication July 20, 1908.

decomposition of hydrogen peroxide into water and oxygen—with a large number of substances, and only in beer yeast was there an exception, the guaiac blueing test not being obtained although the peroxide was readily decomposed. Recently, however, it has been shown that the guaiac blueing reaction is due to a separate enzyme, peroxidase (Spitzer), belonging to the group of oxidases.

Up to the time that Loew published his article on catalase, the property tissues have of decomposing hydrogen-peroxide was supposed to be identical with their power of giving a blue coloration on addition of guaiac in the presence of hydrogen peroxide. Loew definitely proved that these two reactions are distinct, and furthermore that the property of decomposing hydrogen peroxide is likewise due to a specific enzyme of general occurrence. He says, "There is in commerce a kind of diastase, prepared from a fungus (*Aspergillus oryzæ*), the so-called taka diastase, which does not give the reaction for peroxidase, but shows the power of decomposing hydrogen peroxide in a very high degree." Since pure diastase does not show this property, as above mentioned, this diastase preparation must then contain an admixture of another active principle. On the other hand, the trypsin of commerce, prepared from the pancreatic gland, often shows the blue reaction for peroxidase without being able to catalyze hydrogen peroxide. It often contains some peroxidase as an impurity. Furthermore, the filtrate of cultures of *Penicillium glaucum* generally yields no blue reaction with guaiac either for oxidase or peroxidase, but has the power of catalyzing hydrogen peroxide in a high degree. The same is true for cultures of various bacteria.

Tobacco samples of commerce also show the power of catalyzing hydrogen peroxide, frequently to a great extent, although they may have lost the original content of oxidase and peroxidase.

A case where the blue guaiac reactions were obtained in the absence of the power of catalyzing hydrogen peroxide was observed with green tobacco leaves gathered from greenhouse plants in December. The juice was mixed with one-fifth of its volume of absolute alcohol and left for fifteen hours before filtering. Five cubic centimeters of the clear filtrate were mixed with fifteen cubic centimeters of water and five centimeters of hydrogen peroxide,

but no trace of oxygen was given off, while the blue guaiac reactions for oxidase as well as for peroxidase (after killing the oxidase by heating to 70° C.) were obtained with considerable intensity. The property of catalyzing hydrogen peroxide was here observed only with the insoluble parts of the leaf. In the following chapters further instances will be mentioned which prove that these two reactions are produced by different principles.

Since it is clear that the power of catalyzing hydrogen peroxide is not due to any of the known enzymes, it appears justifiable to ascribe this power to a special enzyme. The writers propose to call this, "*catalase.*"

It has long been known that colloidal metals have the power of decomposing hydrogen peroxide, but Loevenhart and Kastle have shown that the action of the organic and inorganic catalyzers is entirely different. Various chemical substances destroy or inhibit the ferment in the tissue extracts, but have no effect upon the metallic decomposition.

Wells in his text-book on Chemical Pathology writes: "Just what function catalase performs is at present merely a matter of speculation." The two possibilities which have suggested themselves to the various workers in this field, are dependent upon the character of the oxygen liberated in the decomposition; whether it is in the form of atomic oxygen, an active oxidizing agent, or molecular oxygen, which is relatively inert. The former is the older view, and in 1899 was revived by Bredig and von Berneck. Traube as early as 1893, described the decomposition of hydrogen peroxide as being due to the molecules decomposing each other mutually. He considered that two weakly held hydrogen atoms of one molecule of the peroxide split a second into two hydroxyl groups, with the result that water was produced and molecular or passive oxygen set free. He represents the equation in this way:

$$\begin{matrix} H & H & H \\ | & | & | \\ O & O & H \end{matrix} + \; O_2 = 2H_2O + O_2.$$

Kastle and Loevenhart in 1903, by a series of very interesting experiments, were able to show that liver catalase is unable to effect the oxidation of neutral potassium oxalate in the presence of

hydrogen peroxide, although the latter was being rapidly decomposed. The work of Traube proved that neutral potassium oxalate is oxidized by the oxygen liberated at the anode in electrolysis (active atomic oxygen), therefore Kastle and Loevenhart concluded that the oxygen liberated by the decomposition of hydrogen peroxide by liver catalase is not atomic but molecular oxygen. Schaffer,[2] in 1905, likewise concluded that when catalase decomposes hydrogen peroxide molecular oxygen is produced. He found that uric acid is oxidized by hydrogen peroxide, but when catalase is present, this oxidation is prevented. According to this, the function of catalase is rather to prevent dangerous forms of oxidation, than to help in normal oxidative processes.

The only work we have been able to find on the catalytic activity of pathological tissues is that of Jolles and Oppenheim. In 1905 they reported a series of cases in which they studied the blood from this point of view. Although they used a very different method from the one here employed, they found, just as we have, that the catalytic activity of the blood is reduced in nephritis and tuberculosis; they found no reduction in diabetes mellitus although one of their cases died in coma, and in this our results are only slightly at variance. By the method they employed only a single observation could be made in each experiment and hence little idea of the velocity of the reaction could be obtained. They allowed the blood to remain in contact with a measured amount of hydrogen peroxide for a definite number of hours; this solution was then acidified with sulphuric acid, and potassium iodide added drop by drop. The hydrogen peroxide which had not been decomposed by the tissue catalase then decomposed the potassium iodide. A known quantity of iodide having been added, the excess was determined by titrating with a solution of sodium hyposulphite of known strength. This, as is readily seen, is a very complex method, but it is still more severely handicapped inasmuch as the rapidity with which the decomposition proceeds cannot be determined. This determination at short intervals seems to be of the greatest possible importance, since in many of our experiments it will be seen, that, although the initial readings were much reduced, at the end of two minutes the maximum reaction had been attained.

[2] Quoted by Wells, Chemical Pathology.

The following method, which is the same as that used by Kastle and Loevenhart, was adopted in all of our experiments, and seems much more satisfactory. In every case the organs were obtained as fresh as possible from the post-mortem examination rooms of the Johns Hopkins Hospital and Bay View Asylum. Ten grammes of each tissue to be examined were carefully weighed off; each portion was placed in a mortar and ground with thoroughly washed white sand; from twenty to thirty cubic centimeters of distilled water were gradually added and the resulting emulsion strained through clean toweling by pressure. The residue was then turned back into the mortar, again ground, more distilled water added, and again strained. This process was repeated once more and the filtrate diluted to one hundred cubic centimeters with distilled water, thus making a ten per cent. cloudy aqueous extract which was preserved with two cubic centimeters of toluine. One cubic centimeter of the cloudy extract thus obtained was added to four cubic centimeters of distilled water in a low, wide-mouthed bottle with a capacity of about one hundred cubic centimeters. A small phial containing five cubic centimeters of neutralized three per cent. commercial hydrogen peroxide was placed in an upright position in the larger bottle, and the latter then connected with a gas burette. The records of the experiments are expressed in terms of cubic centimeters of oxygen liberated by the decomposition of the hydrogen peroxide, readings being made every fifteen seconds covering a period of two minutes. The small phial was readily overturned at the beginning of each experiment, throughout which the larger bottle was constantly and uniformly shaken, thus allowing a thorough mixing of the hydrogen peroxide and the aqueous tissue extract. Two different varieties of hydrogen peroxide were used, and the fact that lead peroxide rapidly decomposes hydrogen peroxide quantitatively was taken advantage of to standardize the different brands[3] of hydrogen peroxide used, as well as to note any change in the activity of a single bottle from time to time. The check was made in much the same manner as the experiments above described. One-half gram of lead peroxide was placed in the larger bottle with five cubic centimeters of water; the phial

[3] Dioxygen, Oakland Chemical Co.; Hydrogen Peroxide, Mallinkrodt Chemical Works.

containing five cubic centimeters of the neutralized hydrogen peroxide to be tested was then placed in the larger bottle. This was connected with the gas burette and shaken for a period of two minutes, during which readings were taken every fifteen seconds. The hydrogen peroxide was entirely decomposed during this time. By this means a rapid and sufficiently accurate method of determining the strength of each bottle of hydrogen peroxide, for further correction, was obtained. The hydrogen peroxide employed was of such a strength that 0.5 gram of lead peroxide liberated about fifty-five cubic centimeters of oxygen in two minutes.

Since the determination of the catalytic activity of the various tissues could not be made until a varying number of hours after the death of the subject, it seemed of the utmost importance to determine what, if any, would be the effect of post-mortem changes. For this purpose a dog was killed and the following experiments performed:

Portions of the lung, liver, kidney and spleen were removed immediately after death, ten per cent. aqueous extracts prepared as described above, and their catalytic activity determined.

Readings were made every fifteen seconds in terms of cubic centimeters of oxygen liberated.

Seconds.	15	30	45	60	75	90	105	120
Kidney	24.6	32	37	42.4	46.4	49.8	52	53.2
Lung	8.4	10	11.6	13	14.4	15.8	16.8	17.8
Liver	28.4	37.2	44.4	51.4	52.6	53	53.4	54
Spleen	3.6	4.6	5.4	6	6.6	7.4	8	8.6

After removing the tissues used in the above determinations ,the animal was sewed up and placed in the ice box (where the bodies are kept before autopsy), for twenty-seven hours. Then pieces of the same organs taken the previous day were removed, and their catalytic activity determined in a similar manner, with the following result:

Seconds.	15	30	45	60	75	90	105	120
Kidney	26.4	34.2	39.4	43.4	46.6	48.6	49.4	50.8
Lung	7	8.4	9.4	10.4	11.2	12	12.8	13.6
Liver	30.6	39.4	44.2	46.4	48.8	49.4	49.6	49.6
Spleen	2.8	3.6	4.4	4.8	5.2	5.6	6	6.2

By a comparison of the above sets of experiments it will be noted that the enzymic activity of the tissues is somewhat lowered by standing in the ice box. In the case of the liver and kidney, although the first few readings were slightly higher, the reduction of the catalytic power was evident at the end.

It seemed desirable to determine any variation in the activity of the enzyme which might be due to the age of the individual from whom the tissues were obtained, but this must be small and relatively unimportant, while the variations due to pathological conditions have been so marked as to overshadow any other possible slight changes. We have been led to this conclusion concerning the activity of catalase at different ages from the fact that in case No. 3, a still-born infant which showed no pathological lesion, the tissues had a relatively high catalytic activity, while in several old subjects, in spite of some slight pathological condition, which would tend to lower the catalytic activity, there was no appreciable decrease. Case No. 17 is an illustration of this statement.

There are in this series thirty-one cases to be reported. These are divided into several groups. At the beginning of each group is a short abstract of the clinical history and the post-mortem findings of each case. When such data could be obtained, the number of red blood cells, the percentage of hæmoglobin, the condition of the urine, and a few brief important points in the anatomical diagnosis are included.

NEPHRITIS.

Case No. 1.—Autopsy No. 2971. White male, 33 yrs. Died 8 A. M., Dec. 8. Autopsy 9.30 A. M., Dec. 8, 1908.

Clinical Diagnosis—Chronic nephritis, uræmia, tuberculosis, albuminuric retinitis.

R. B. C.—3,800,000; W. B. C.—10,000; Hb.—58 per cent.

Urine—Sp. gr. 1015. Reaction—acid. Albumin—3.8 gm. per L.

Microscopical Examination—R. B. C., W. B. C., granular and waxy casts.

Blood Pressure—200 to 210.

Anatomical Diagnosis—Chronic diffuse nephritis, pale granular kidneys; chronic pulmonary tuberculosis; anæmia and emaciation. In the lungs a few small caseous nodules are found which show microscopically an exudate into the surrounding alveoli. Spleen is congested; the venules show slightly thickened walls. *Kidneys* weigh 250 gm.; they show a marked increase of fibrous tissue and extensive epithelial changes.

Case No. 7.—Autopsy No. 2977. Colored male, 75 yrs. Died 4 P. M., Dec. 15. Autopsy 10 A. M., Dec. 16.

Clinical Diagnosis—Chronic nephritis, arterio-sclerosis, emphysema.

R. B. C.—3,500,000; W. B. C.—10,380; Hb. 60 per cent.

Urine—Sp. gr. 1012. Reaction—acid. Albumin—trace.

Microscopical Examination—Finely granular casts, epithelial cells.

Anatomical Diagnosis—Chronic diffuse nephritis; arterio-sclerosis; senile

spleen; emphysema. Liver shows a slight amount of fatty degeneration and jaundice. *Kidneys* weigh 260 gm; there is only a slight amount of interstitial change, but extensive parenchymatous degeneration.

Case No. 9.—Autopsy No. 2991. White female, 25 yrs. Died 2 A. M., Jan. 8. Autopsy 11 A. M., Jan. 8.

Clinical Diagnosis—Chronic nephritis; uræmia; broncho-pneumonia.

R. B. C.—3,896,000; W. B. C.—10,600; Hb. 75 per cent; blood pressure 210 to 240.

Urine—Sp. gr. 1010. Reaction—acid. Albumin—1 gm. per L.

Microscopical Examination—Hyaline and granular casts.

Anatomical Diagnosis—Chronic diffuse nephritis, small granular kidneys; cerebral hæmorrhage; broncho-pneumonia; chronic splenic tumor; emaciation. *Kidneys* weigh 120 gm.; the cortex measures 2–4 mm. in thickness, and is broken up by coarse bands of fibrous tissue.

Case No. 10.—Autopsy No. 2992. White male, 60 yrs. Died 6 P. M., Jan. 12. Autopsy 11 A. M., Jan. 13.

Clinical Diagnosis—Acute exacerbation of chronic nephritis; arterio-sclerosis.

R. B. C.—4,100,000; W. B. C.—10,000; Hb. 94 per cent.

Urine—Sp. gr. 1013. Reaction—acid. Albumin—2 gm. per L.

Blood Pressure—240.

Anatomical Diagnosis—Acute and chronic diffuse nephritis; broncho-pneumonia; pulmonary œdema and emphysema; chronic passive congestion of spleen. *Kidneys* weight 320 gm.; the capsule is thickened and strips with difficulty, leaving a granular surface; the striations are irregular and obscured, and there are many small hæmorrhages in the cortex. Microscopically there are red blood cells and polymorphonuclear leucocytes in the tubules, the epithelial elements are degenerated, and the glomeruli are changed into fibrous bodies.

Case No. 11.—Autopsy No. 2996. Colored female, 46 yrs. Died 11 P. M., Jan. 16. Autopsy 11 A. M., Jan. 17.

Clinical Diagnosis—Acute lobar pneumonia; acute endocarditis; cerebral thrombosis; multiple infarcts in brain, lungs, etc.; pulmonary abscess; chronic nephritis; aortic insufficiency.

R. B. C.—3,744,000; W. B. C.—21,600; Hb. 90 per cent.

Urine—Sp. gr. 1010. Reaction—acid. Albumin—1.2 gm. per L.

Microscopical Examination—Granular and hyaline casts; R. B. C.

Blood Pressure—100 to 150.

Anatomical Diagnosis—Puriform softening of auricular thrombus; infarction of brain, spleen, kidney, and lung; lobar pneumonia with gangrene; chronic diffuse nephritis. *Kidneys* weigh 320 gm. and show extensive degenerative changes.

Case No. 13.—Autopsy No. 2999. White male, 25 yrs. Died 12 noon, Jan. 21. Autopsy 2 P. M., Jan. 21.

Clinical Diagnosis—Chronic interstitial nephritis; cirrhosis of liver; uræmia.

R. B. C.—6,430,000; W. B. C.—14,000; Hb. 58 per cent.

Urine—Sp. gr. 1013. Reaction—acid. Albumin—3 gm. per L.

Microscopical Examination—Hyaline, waxy and granular casts, R. B. C., W. B. C. and renal epithelial cells.

Blood Pressure—220.

Anatomical Diagnosis—Chronic diffuse nephritis; broncho-pneumonia; diffuse cirrhosis of the liver, with enlargement of the spleen. *Kidneys* weigh 220 gm.; the cortex varies from 2 to 4 mm. in thickness; there is an increase in cellular connective tissue and a marked degeneration of the parenchyma.

Case No. 14.—Autopsy No. 3002. Colored male 45 yrs. Died 2 A. M., Jan. 25. Autopsy 10 A. M., Jan. 25.

Clinical Diagnosis—Chronic nephritis, arterio-sclerosis.

R. B. C.—4,500,000; W. B. C.—8,750; Hb. 53 per cent.

Urine—Sp. gr. 1015. Reaction—acid. Albumin 0.75 gm. per L.

Microscopical Examination—Granular, hyaline, and epithelial casts, and W. B. C.

Anatomical Diagnosis—Chronic diffuse nephritis; broncho-pneumonia; pulmonary œdema; infarcts of lung and spleen; thrombi in right auricular appendage, and in apex of left ventricle; emphysema. *Kidneys* weigh 300 gm.; the cortex measures 4 mm., the striations are obscured by dense bands of connective tissue, and the epithelium shows extensive degeneration.

Case No. 19.—Autopsy No. 3024. Colored male, 53 yrs. Died 9 P. M., Mar. 11. Autopsy 10 A. M., Mar. 12.

Clinical Diagnosis—Chronic nephritis; arterio-sclerosis.

R. B. C.—4,320,000; W. B. C.—5,400; Hb. 75 per cent.

Urine—Sp. gr. 1010. Reaction—acid. Albumin—1 to 3 gm. per L.

Microscopical Examination—Granular and hyaline casts, epithelial and pus cells.

Blood Pressure—220 to 245.

Anatomical Diagnosis—Chronic diffuse nephritis; arteriosclerosis; chronic passive congestion of lungs and spleen; emphysema. *Kidneys* weigh 340 gm.; the cortex is thin, and the striations distorted.

Case No. 26—Bay View Autopsy No. 136. White male, 45 yrs. Died 5 P. M., Dec. 10. Autopsy 9 A. M., Dec. 11.

Anatomical Diagnosis—Chronic diffuse nephritis (large pale kidney); lobar pneumonia; diffuse cirrhosis of liver; acute splenic tumor.

Case No. 30.—Bay View Autopsy No. 140. White male, 68 yrs. Died 12 P. M., Dec. 17. Autopsy 11 A. M., Dec. 18.

Anatomical Diagnosis—Chronic diffuse nephritis; chronic splenic tumor; emphysema.

KIDNEY—Readings made every fifteen seconds, expressed in terms of cubic centimeters of oxygen.

Seconds.	15	30	45	60	75	90	105	120
Case No. 1.....	1	1.2	1.7	1.8	2.2	2.4	2.5	2.8
Case No. 7.....	9.6	12	14.2	16	18.4	21.4	23.8	26.6
Case No. 9.....	7.2	8.8	10.2	11.8	12.4	15	16.4	18
Case No. 10.....	10.6	13.8	16.2	18.4	21	24	26.6	28.4
Case No. 13.....	4.4	5.6	7	8.2	9.2	10.4	11.8	13
Case No. 14.....	12.4	16	19.2	23	26.6	30.2	33.8	34
Case No. 19.....	8.8	11.4	13.8	16	18.2	20.6	23	25.4
Case No. 26.....	4.6	5.4	8.8	11	11.6	13.2	14.8	14.8
Case No. 30.....	7	10.6	13.8	16.8	19.6	22	26.8	31.4

In all these cases the nephritis was severe and might be considered as the ultimate cause of death. In Cases Nos. 1, 9 and 13, the clinical diagnosis of uræmia was made, and it will be noted that the readings in these are remarkably low. In all of the other cases the reduction seemed to be in direct proportion to the intensity of the anatomical picture as well as to the clinical findings.

LUNG.

Seconds.	15	30	45	60	75	90	105	120
Case No. 1	4.4	5.6	6.6	7.4	8.4	9.4	10.4	11.4
Case No. 7	10.8	12.4	14.2	16	18.2	20.4	22.8	25.6
Case No. 9	21	25.4	28.4	31.4	34.6	38	41	44.2
Case No. 10	7	9	10	12.2	13.6	15	16.8	18.2
Case No. 13	11.4	14.8	18.6	21.8	25.4	28.5	32.8	36.2
Case No. 14	30	38.8	45.6	50.6	53	53.6	54	54
Case No. 19	25.4	32.6	38.4	43.4	47.8	50.8	52.2	53.8
Case No. 26	16.8	20	24.4	27	30.8	34.6	37.4	39.4
Case No. 30	5.6	7.6	10.2	12.2	14	16.4	18.8	21.4

In Cases Nos. 1, 7 and 30, the lungs were in good condition, and showed neither pneumonia, nor congestion. In these it will be seen that the catalytic activity is low. In the remainder of the cases either pneumonia or congestion was present, and it will be shown later that in these conditions there is an increased catalytic activity. Despite the presence of pneumonia, however, in the cases in which the nephritis is more severe, the activity of the lung is reduced.

LIVER.

Seconds.	15	30	45	60	75	90	105	120
Case No. 7	11.2	14.4	16.2	22	26.2	30.4	34.8	39.6
Case No. 9	34	43	47.4	50.2	51.2	51.6	51.6	51.8
Case No. 10	18	23.8	29.4	34.2	39	44.8	49.8	53
Case No. 13	35.4	43	47.2	48.4	48.8	49	49.2	49.4
Case No. 14	31	37.8	42.8	45.6	47	47.4	47.6	47.6
Case No. 19	30.8	41.6	48.4	52.2	54	54.4	54.6	54.8
Case No. 26	7.1	9.4	12.2	14.8	17.6	20.3	23.6	26.6
Case No. 30	16.8	26.2	31.6	37.8	44	48	50.2	51.6

Except in Cases No. 7, 10, 26 and 30 there is not a remarkable reduction in the catalytic activity of the liver. Of these, Cases Nos. 10 and 30 reach at the end what might be considered a maximum reading, even though the initial reading is low. It seems possible that in many cases in which the first two or three read-

ings are much reduced, while a maximum decomposition is reached at the end, the inhibiting factor may be overcome during the experiment. The explanation of the extremely low readings in Cases Nos. 7 and 26 is at present not clear.

SPLEEN.

Seconds.	15	30	45	60	75	90	105	120
Case No. 9	20.4	25	29.4	32.6	36.4	40.8	44.2	47.4
Case No. 10	25	32	38	42.6	46	48.6	50.2	51.2
Case No. 13	28.4	35.6	43	49.2	52.6	54	54.6	55
Case No. 14	30	37.6	44	49.4	53	54.6	55.4	55.4
Case No. 19	50.6	52.4	53	53.2	53.4	53.6	53.8	54
Case No. 26	6.7	8.8	10.8	12.8	14.6	16.8	19	21.2
Case No. 30	22.8	31	37.2	44	46.4	40.4	51	51

The variations in the readings of the spleen in the above cases seem to be due most probably to the amount of blood in the organ rather than to the severity of the associated nephritis.

BLOOD.

Seconds.	15	30	45	60	75	90	105	120
Case No. 9	46	51.4	52.4	52.6	53	53	53	53
Case No. 10	32	41	45.4	47.6	49	49.8	50.4	51
Case No. 11	38.4	47	50.6	52.2	52.6	52.8	53	53
Case No. 13	22	30	37	42	46	47.8	48.2	48.2
Case No. 14	26	36.6	46.4	49.6	53.2	54	54.2	54.4
Case No. 26	15	18	20.8	23.2	25.8	28.6	31	33.6

With normal blood the maximum reaction is reached at the first reading. The blood in the above cases was obtained in a fluid condition from the large vessels at the time of autopsy. It is very important to obtain the blood before clotting has taken place, because, as Bergengrun has shown, the catalase is carried in the stroma of the red blood cells, and not in the hæmoglobin or in the serum. This has been confirmed by us. The clot would naturally be much more active on account of its greater content of red blood cells, and it is, therefore, most necessary to obtain the blood before any abnormal distribution of its elements has occurred. The readings of the blood from the cases of nephritis show a marked reduction when compared with the other conditions in which the blood was examined.

ECLAMPSIA.

The blood in the experiments to be quoted was obtained from two cases of eclampsia in the Maternity Ward, in which venesection was performed as a therapeutic measure.

Seconds.	15	30	45	60	75	90	105	120
Case A	52.6	52.8	52.8	53	53.2	53.4	53.4	53.6
Case B	51.2	51.4	51.8	52	52.2	52.4	52.6	52.8

The reaction practically reached the maximum in the first fifteen seconds. It is interesting to compare these determinations with those obtained from the blood in the above series of nephritis for this difference may prove of some value clinically in the differential diagnosis between eclampsia and chronic nephritis. Blood has been obtained from cases of chronic nephritis in the wards and tested for its catalytic activity with the result that in every case it has shown a reduction. We have so far been unable to confirm further the results found in eclampsia since the cases have not been forthcoming. By diluting the blood 1–400 with distilled water, we find that the end reaction is reached much more gradually than when stronger solutions of blood are used, and therefore, the differences between normal and nephritic blood stand out in greater contrast. The contrast is likewise more sharply brought out when readings are made every five seconds during the first fifteen seconds.

In summing up the changes which are apparent in the cases of chronic nephritis studied, it will immediately be seen that the most marked changes, evidenced in the lowering of the catalytic activity, are in the kidney itself. Here the reduction seems to be in direct proportion to the extent of the lesion. In the one example of large pale kidney (Case No. 26) the reduction is so marked not only in the kidney itself, but in the blood and other organs as well, that it seems possible that there is in this type a greater decrease in the catalytic activity than in the small contracted kidney. In all cases of nephritis, however, there is not only a reduction in the catalytic activity of the kidney, blood and other organs, but the acid urine of several nephritics, when used to dilute an active extract of tissue catalase, has shown a much greater inhibiting action than normal acid urine. The following experiment illustrates this statement:

Urine was obtained from Case No. 9, two weeks before death, and was used in connection with a 10 per cent. aqueous extract of a haemorrhagic pulmonary infarct from Case No. 2. Normal urine was used in a check experiment.

1. 1 c.c. of the above 10 per cent. aqueous extract + 4 c.c. water + 5 c.c. hydrogen peroxide.

Seconds.	15	30	45	60	75	90	105	120
	50	50.2	50.6	50.8	51.2	51.4	51.6	51.8

2. 1 c.c. of the above 10 per cent. aqueous extract + 4 c.c. normal urine + 5 c.c. hydrogen peroxide.

Seconds.	15	30	45	60	75	90	105	120
	26	32.8	37.6	40.4	43.6	45.4	46.8	48

3. 1 c.c. of the above 10 per cent. aqueous extract + 4 c.c. nephritic urine + 5 c.c. hydrogen peroxide.

Seconds.	15	30	45	60	75	90	105	120
	4.4	10.4	17.4	26.8	36	47.4	51.8	54

Catalase is much more active in a slightly alkaline medium while in a markedly acid medium its activity is not reduced. The above urines were both acid but the total acidity was not determined. In several subsequent observations with nephritic urine which was alkaline the catalytic activity was greater than with normal urine. It is necessary, therefore, to have some check on the results by a quantitative estimation of the acidity, for it is possible that the reaction of the urine might be responsible for the above results. On the other hand, it has occurred to us that the kidney may not be entirely a passive element in nephritis, but that it may secrete some substance which is harmful to the organism, and which finds its way into the circulation as well as the urine, and manifests itself by a reduction in the catalytic activity.

PNEUMONIA.

Case No. 4.—Autopsy No. 2974. White infant, 2 days old. Died Dec. 9. Autopsy Dec. 11.

Pregnancy and labor were normal.

Anatomical Diagnosis—Broncho-pneumonia; uric acid infarcts of kidney; fatty degeneration of the liver. Microscopically small patches of pneumonia in the stage of red hepatization were seen. The fatty degeneration of the liver was very extensive.

Case No. 5.—Autopsy No. 2975. Colored male, 15 years. Died 8 P. M., Dec. 13. Autopsy 10 P. M., Dec. 13.

Clinical Diagnosis—Tuberculosis of the vertebræ; terminal pneumonia.

Urine—Sp. gr. 122. Reaction acid. Albumin—negative. Sugar—negative. Microscopical Examination—negative.

Anatomical Diagnosis—Broncho-pneumonia; parenchymatous degeneration of liver and kidney; tuberculosis of the vertebræ; emaciation. The left lung showed fresh areas of broncho-pneumonia. The right lung was normal throughout.

Case No. 22.—Bay View Autopsy No. 132. White male, 42 years. Died Dec. 4, 6 A. M. Autopsy Dec. 4, 12 A. M.

Anatomical Diagnosis—Broncho-pneumonia; pulmonary œdema; aortic insufficiency; chronic passive congestion of the liver, spleen and kidneys; fatty degeneration of the liver.

Case No. 8.—Autopsy No. 2979. White male, 46 years. Died 9 A. M., Dec. 19. Autopsy 1 P. M., Dec. 19.

Clinical Diagnosis—Alcoholic cirrhosis of the liver; ascites; acute pleuro-pericarditis.

R. B. C.—4,500,000; W. B. C.—12,000; Hb. 72 per cent.

Urine—Sp. gr. 1020. Reaction acid. Albumin and sugar—negative. Microscopic Examination—negative.

Blood pressure—115.

Anatomical Diagnosis—Acute broncho-pneumonia with abscess formation; cirrhosis of the liver; chronic pancreatitis; chronic splenic tumor. The liver cells show a high degree of fatty change. The pneumonia is in the stage of gray hepatization.

(Refer to Cases 9, 10, 13, 14, 11, 18, 26, 23.)

LUNG.

Seconds.	15	30	45	60	75	90	105	120	
Case No. 4....	32.4	40.4	43.8	45.6	46.4	47.4	48.4	49.6	
Case No. 5....	39.6	43.4	44.3	45.2	46.2	46.8	48	50	(Pneumon.)
Case No. 5....	15.4	19.4	23.4	27.6	32.2	37.2	40.8	45.6	(Normal.)
Case No. 8....	20.2	25.4	30	34.4	39	42.4	46	48.8	(Gray hep.)
Case No. 8....	27.4	34	41	48	48.8	50.4	50.4	50.4	(Normal.)
Case No. 22....	29.8	39.8	45	47.8	49.2	49.6	48.8	50.4	

In every case the pneumonic lung shows an increased catalytic action, which is probably due to the presence of the red blood cells in the exudate. In Case No. 5, the first reading was taken from an extract of the lung which showed an early stage of broncho-pneumonia, the red blood cells being everywhere well preserved, while the second reading was taken from an extract of lung which was everywhere apparently normal, except for a slight anæmia which was present throughout the tissues. The difference is remarkable, the initial reading in the pneumonic lung being 34.6 while in the uninvolved lung the initial reading was only 15.4 and the following ones correspondingly low. An interesting contrast was found in Case No. 8, in which the first reading was made from lung tissue in the stage of late gray hepatization and showed no increased activity, while the second reading was taken from the uninvolved lung which was slightly congested and consequently had a slightly greater activity. Among the cases of nephritis there

were several instances in which the pneumonia was the terminal event. Among these, Cases Nos. 9 and 14 are high in comparison to the readings in those cases where there was nephritis without pneumonia, while they are comparatively low for the cases uncomplicated by nephritis. In Cases Nos. 10, 13 and 26, however, despite the presence of the pneumonia, the readings are low, no doubt due to the marked decrease of activity accompanying the kidney lesion.

In connection with the cases of pneumonia it will be interesting to consider the following case:

Case No. 2.—Autopsy No. 3009. Colored male, 66 yrs. Died 8 A. M., Jan. 31. Autopsy 12 M., Jan. 31.

Anatomical Diagnosis—Thrombosis of the left ventricle; hæmorrhagic infarcts in lungs; anæmic infarcts in spleen and kidneys; chronic passive congestion of lungs and kidneys.

Seconds.	15	30	45	60	75	90	105	120	
Blood	53.8	54.4	54.4	54.6	54.6	54.6	54.6	54.6	
Lung (2)	50	50.2	50.6	50.8	51.2	51.4	51.6	51.8	(Infarct)
Lung (1)	21.6	29.4	34	37.8	41.4	44.8	47.6	50	(Normal)
Kidney	19	27.4	30.4	34.8	37.4	41.4	43.6	46.2	

Here lung (1) was apparently normal, and gave readings which correspond with what is considered normal. In lung (2) however, the extract was made from a typical fresh hæmorrhagic infarct, with the result that the reaction practically reached the maximum at the initial reading. This case is cited to give strength to the conclusion that the increased catalytic activity in pneumonia and congestion, is very likely due, for the most part, if not entirely, to the presence of an increased number of intact red blood cells.

LIVER.

Seconds.	15	30	45	60	75	90	105	120
Case No. 4	17.6	22.4	28	33.6	38.8	42.4	45	46.2
Case No. 5	38.2	43.4	44.6	45.2	45.8	46.2	46.2	47
Case No. 8	8.4	11.6	14.4	17.8	21	24.4	27.6	31.2
Case No. 22	18.4	22.4	26.4	36.6	33.2	35.8	38.6	41

In Case No. 4 the low reading can probably be attributed to the extensive fatty infiltration and likewise in Case No. 8 there was an old alcoholic cirrhosis, the remaining liver tissue showing marked fatty change. Case No. 22 showed red atrophy, and although there were numerous disintegrated red blood cells about the hepatic vein, these had probably long since lost their catalase.

SPLEEN.

Seconds.	15	30	45	60	75	90	105	120
Case No. 4	19.4	25	27.8	36.8	42	45.4	48.6	48.6

KIDNEY.

Seconds.	15	30	45	60	75	90	105	120
Case No. 4	9.2	11.8	13.6	16.8	19.4	22.4	25.2	28.2
Case No. 5	15.6	20.8	25	29.6	33.8	38.8	43	47.6
Case No. 8	18.8	24.2	29.6	34	39.2	44	47	48.8

In Case No. 4 the uric acid infarcts might account for the low reading, while in Case No. 5 there was marked parenchymatous degeneration and anæmia.

BLOOD.

Seconds.	15	30	45	60	75	90	105	120
Case No. 8	51.2	—	—	—	—	—	—	51.2

This reading stands out in marked contrast to those of the blood in the cases of nephritis, the decomposition being complete during the first fifteen seconds.

In this series the only results of interest are found in the lungs. Extracts made from the involved areas of broncho-pneumonia in the stage of red hepatization invariably showed an increased catalytic activity, which was evidently due merely to the presence of the well preserved red blood cells in the exudate, for in the stage of gray hepatization there was no increase and in the fresh hæmorrhagic infarct the decomposition reached a maximum in the first fifteen seconds. The blood and other tissues examined showed no increased activity. The conclusion is, therefore, justified that there is no new formation of catalase, and no formation of any substance which would increase the action of the enzyme in the body.

TUBERCULOSIS.

Case No. 16.—Autopsy No. 3006. Colored male, 3 mos.

Anatomical Diagnosis—General miliary tuberculosis; caseous lobar pneumonia; anæmia and emaciation.

Case No. 21.—Bay View Insane. Colored female, 23 yrs. Died 6 P. M., Dec. 7. Autopsy 11 A. M., Dec. 8.

Anatomical Diagnosis—Broncho-pneumonia; tuberculosis of the liver and spleen; congestion of the kidneys. The body was fairly well nourished. There was extensive tuberculosis of the lesser peritoneum with cavities in the liver, and tuberculous ulceration of the stomach.

Case No. 24.—Bay View Autopsy No. 134. White male, 32 yrs. Died 9 P. M., Dec. 8. Autopsy 3 P. M., Dec. 9.

Anatomical Diagnosis—Acute pulmonary miliary tuberculosis; acute splenic tumor; fatty degeneration of the liver (slight); congestion of the kidneys. The lung tissue between the miliary tubercles was deeply congested.

Case No. 27.—Bay View Autopsy No. 137. White male, 73 yrs. Died 7 P. M., Dec. 12. Autopsy 11 A. M., Dec. 13.

Anatomical Diagnosis—Chronic pulmonary tuberculosis with cavity formation; miliary tuberculosis of the spleen and liver; acute splenic tumor; chronic passive congestion of the liver and kidneys; fatty degeneration of the liver.

Case No. 25.—Bay View No. 135. White male, 37 yrs. Died 3 A. M., Dec. 11. Autopsy 12 M., Dec. 11.

Anatomical Diagnosis—Tuberculous caseous pneumonia with cavity formation.

Case No. 28.—Bay View No. 138. White male, 32 yrs. Died 2 A. M., Dec. 13. Autopsy 12 M., Dec. 13.

Anatomical Diagnosis—Chronic pulmonary tuberculosis; chronic diffuse nephritis; emaciation.

Case No. 29.—Bay View No. 139. Colored male, 24 yrs. Died 4 P. M., Dec. 15. Autopsy 4 P. M., Dec. 16.

Anatomical Diagnosis—Acute tuberculous broncho-pneumonia with cavity formation; miliary tuberculosis of the liver and spleen; emaciation.

LUNG.

Seconds.	15	30	45	60	75	90	105	120
Case No. 16	13.6	17.4	20.2	22.4	24.6	26.8	29.8	32
Case No. 21	7.2	9.2	10.4	11.8	13	14.2	15.6	16.8
Case No. 23	16.6	19.8	25.6	30	34	39.2	40.2	43
Case No. 24	30	36.8	43.6	44	45.4	46.4	46.8	46.8
Case No. 27	12	15.4	18.2	21	24.8	29	32.8	36.6
Case No. 25	11.8	15.8	19.6	23.4	26.8	30.4	33.4	38
Case No. 28	6.4	7.8	9.2	10	11.4	12.8	14	15.4
Case No. 29	5.8	7.2	8.4	9.6	10.8	12.4	13.8	15.8

It will be seen that there is a uniform and marked reduction of the power of the tuberculous lung to decompose hydrogen peroxide. In Case No. 24 the reading is high, but the lung in the gross was voluminous, very deep red on section, and studded everywhere with minute tubercles. Microscopically, the tissue between the tubercles was greatly engorged with well preserved red blood cells.

LIVER.

Seconds.	15	30	45	60	75	90	105	120
Case No. 16	23.4	29.6	33.8	37.8	44	45.8	47.8	48.8
Case No. 21	21.8	28.8	35.4	38.6	40	43.4	45	46
Case No. 23	19.6	27.4	32	36.6	40.8	43.8	45.6	46.2
Case No. 24	13.4	15.6	21.2	24.8	28.4	32.2	35.6	39
Case No. 27	12	15.4	20.2	25.4	30.6	36	42.2	46.8
Case No. 29	15	20.6	26.2	32.6	38.6	42.4	47.6	53.4

In all of the cases there is a slight but definite decrease in the catalytic activity. The liver showed a varying amount of involvement, from microscopic tubercles to cavities.

SPLEEN.

Seconds.	15	30	45	60	75	90	105	120
Case No. 16	23	31.4	36.2	40	43.2	46	48	49.6
Case No. 21	31.8	38.8	41.6	42.6	43.2	43.6	—	—
Case No. 23	22.8	30	34.4	37.4	39.8	41.8	43	44.2
Case No. 27	20.8	29.2	36	41.6	45.6	48	49.4	51.2

In comparison with the other diseases the readings of the spleen in tuberculosis are low, except in Case No. 21 where, although there was an extensive involvement, the splenic tissue between these areas was much engorged.

KIDNEY.

Seconds.	15	30	45	60	75	90	105	120
Case No. 16	11.8	15.4	18	20	22	24.2	26.2	28.2
Case No. 21	10.4	20.4	24.4	27.6	30.8	33.8	37.4	38.4
Case No. 24	21	30.2	35.4	39.4	41.4	42.6	43.4	44
Case No. 27	13.6	18.2	22.4	26.8	31.4	36.2	41.8	45.6
Case No. 29	15	21.8	26	30.4	36.6	42	46.4	49.8

Here also the readings are low, except in Case No. 24, where the kidneys were swollen and congested.

It is seen that in these cases of tuberculosis the catalytic activity of the lungs is most markedly reduced. This is probably in large part due to the absence of blood in the tuberculous areas. On the other hand, the reaction of the liver, spleen, and kidneys is lowered, and it would hardly be possible to attribute this to the presence of tuberculous change in these organs, but rather to the general anæmia which accompanies the process. Whether this is sufficient to account for the reduction, or whether the tuberculous process is the seat of formation of some inhibiting substance has not been determined.

JAUNDICE.

Case No. 17.—Autopsy No. 3021. White male, 59 years. Died 1 A. M., Feb. 23. Autopsy 10 A. M., Feb. 23.

Clinical Diagnosis—Carcinoma of the common bile duct; acute gangrenous cholecystitis. Operation Feb. 11—cholecystostomy followed by diffuse bronchitis and broncho-pneumonia.

R. B. C.—4,362,000; W. B. C.—17,400; Hb. 85 per cent.

Urine—Sp. gr. 1018. Reaction—acid. Albumin—trace. Sugar—negative.

Microscopic Examination—Bile stained granular casts.

Anatomical Diagnosis—Carcinoma of the common bile duct; metastasis to the liver; jaundice; cirrhosis of the liver; broncho-pneumonia; chronic diffuse nephritis.

Kidneys weigh 370 gm. The cortex averages 6 mm. in thickness.

Seconds.	15	30	45	60	75	90	105	120
Kidney	26.6	36	41.6	45.4	48	50	51.2	51.8
Spleen	39	46.8	49.6	50.6	51	51.2	51.4	51.4
Liver	29.6	39.2	44.8	47.4	49.8	50.8	51.2	51.6
Lung	23	28.4	32	35.4	38.2	41.4	44	46.2
Blood	48.4	50	50.4	—	—	—	—	50.4

This case of jaundice is quoted to show that, in spite of a small amount of nephritis, there is no decrease in the catalytic activity as suggested by Jolles and Oppenheim. They report a case of catarrhal jaundice with gall stones in which they found a minimal catalytic activity. It seems probable that the mere jaundice in their case could not have accounted for the low catalytic activity, since in Case No. 17 of our series there was extreme jaundice but no reduction in the catalytic activity.

DIABETES MELLITUS.

Case No. 20.—Autopsy No. 3026. White female 19 yrs. Died 12 M. March 18. Autopsy 3 P. M., March 18.

Clinical Diagnosis—Diabetes mellitus; gangrene of left forearm.

R. B. C.—5,472,000; W. B. C.—21,160; Hb. 101 per cent.

Urine—Sp. gr. 1027. Reaction—acid. Albumin—trace. Sugar—3 per cent.

Microscopical Examination—Hyaline and granular casts; epithelial cells. Blood pressure—120.

Anatomical Diagnosis—Gangrene of arm; fatty liver; epithelial necrosis of kidneys; œdema and hyperæmia of lungs. *Kidneys* weigh 430 gms. Parenchyma is yellow and granular.

Seconds.	15	30	45	60	75	90	105	120
Lung	16.6	20.4	23.4	26.6	29.6	33	36.6	39.4
Kidney	11.4	14.4	17.6	20.6	24	27.4	30.4	37
Liver	48.6	53.2	53.8	54.2	54.4	54.6	54.8	54.8

In this case, the lung and kidney show a definite reduction in their catalytic activity. The kidneys, however, showed extensive epithelial necrosis which might account for their low activity. The liver gives a high reading, even though it showed fatty infiltration. This might be explained by the extensive hyperæmia it showed in places. The pancreas was apparently normal both grossly and microscopically.

TOXÆMIA OF PREGNANCY.

Case No. 18.—Autopsy No. 3023. White female, 27 yrs. Died 8 P. M., March 10. Autopsy 10 A. M., March 11

Clinical Diagnosis—Toxæmia of pregnancy. History of operative labor (low forceps); febrile puerperium.

Urine—Sp. gr. 1028. Reaction—acid. Albumin—heavy precipitate. Sugar—negative.

Microscopical Examination—Hyaline casts.

Anatomical Diagnosis—Broncho-pneumonia; acute splenic tumor; fatty degeneraton of the liver, kidney and myocardium; epithelial necrosis of the kidney; pulmonary œdema.

Seconds.	15	30	45	60	75	90	105	120
Lung	23.6	29.6	35	40	45	49.2	50.4	51.8
Liver	44	50.2	51.2	51.4	51.4	51.6	51.8	51.8
Spleen	48	52.4	53.4	53.6	53.8	54	54.2	54.4
Kidney	17	22	27.4	32.2	37	41.4	45.6	48.6

The lungs, though showing early broncho-pneumonia, are only slightly increased in their activity; the urine contained albumin and hyaline casts, and the kidneys showed extensive epithelial degeneration. The high reading of the spleen is probably due to its congestion.

INFANTS.

Case No. 3.—Autopsy No. 2973. White male, still born. Birth 12 M., Dec. 6. Autopsy 12 M., Dec. 9.

Clinical History—Pregnancy complicated by placenta præva. Operative delivery; dilatation of cervix (Harris), version and extraction. Febrile puerperium. Child still-born; macerated.

Anatomical Diagnosis—No cause of death could be found.

Case No. 6.—Autopsy No. 2976. White male, 2 days. Died 7 P. M., Dec. 13. Autopsy 4 P. M., Dec. 14.

Clinical History—Operative delivery, low forceps; febrile puerperium.

Clinical Diagnosis—Congenital syphilis.

Anatomical Diagnosis—Congenital syphilis.

Case No. 12.—Autopsy No. 2998. White male, 8 days. Died 6 A. M., Jan. 19. Autopsy 11 A. M., Jan. 21.

Clinical History—Premature delivery by *accouchment forcé* for eclampsia. Child was taken to mother to nurse perfectly well, and ten minutes later was found dead with its head and face cyanotic.

Anatomical Diagnosis—No cause of death could be found.

CASE No. 3.

Seconds.	15	30	45	60	75	90	105	120
Lung	26.5	33.4	38.2	41.6	43.8	46.2	47.2	47.8
Liver	19.8	27.6	33.8	39.2	42.2	44.4	46.4	47
Kidney	11.4	15.4	18.2	21.8	25	28	31.6	34.8

CASE No. 6.

Lung	7.6	9.4	11.2	12.4	14.4	16.2	18.4	19.8
Liver	16.8	22	25.8	30.2	34.8	41.4	44.4	46.8
Spleen	22.8	29.4	34.8	40.2	44	47.2	49.2	49.2
Kidney	6.4	7.8	9.2	10.2	11.8	13.4	14.6	15.8

CASE No. 12.

Lung	19	24	29	35	40.8	45.4	49.2	52.2
Liver	23	32.6	39.8	46.2	50.6	53.6	55.4	56.2
Spleen	22.4	28.6	34	39	44	49	52.8	55
Kidney	7.4	11	14.6	18	19.8	24	28.4	32.6
Blood	51	51.4	51.8	52.2				52.4

In the case of congenital syphilis, Case No. 6, the lung, liver, and kidney extracts give a lower catalytic reaction than the corresponding tissue extracts from Cases Nos. 3 and 12. The autopsy in Case No. 6 was done twenty hours after death, that in Case No. 3, three days after death, and in Case No. 12, two days after death. In consideration of the fact that tissues lose, to some extent, their catalytic activity through post-mortem changes, it is probably true, that the difference between Case No. 6 and Cases Nos. 3 and 12, would have been greater had they been done at equal intervals after death. That Case No. 3 was still-born, and showed considerable catalytic activity, is worthy of note.

CARBON MONOXIDE POISONING.

CASE No. 15.

Seconds.	15	30	45	60	75	90	105	120
Blood	22.6	32.6	37.2	40.6	44.4	45.4	46.6	47.6
Serum	2.6	3.6	4	4.4	5	5.6	6	6.4

The blood was obtained from this case post-mortem, though no other tissues were examined, as an autopsy could not be obtained. Jolles and Oppenheim say that there is only a very slight reduction in the enzymic activity of the blood after passing carbon monoxide through it. The reduction is very marked in the one case of carbon monoxide poisoning here reported.

From the above results, it is impossible for us to agree with Jolles and Oppenheim in their theory, that in the group of diseases which are characterized clinically by coma, such as, diabetes mellitus, nephritis, icterus, eclampsia, there is retained in the blood a catalytic inhibiting substance, and that the symptoms are due not

to the retention of specific substances in the blood, but to the lack of oxidation in the tissues. That there is a decreased catalytic activity in the blood in nephritis, is quite evident, but in both their cases of diabetes mellitus and in our one case, and in our cases of eclampsia and jaundice, the catalytic activity was not reduced. Although these diseases may be allied clinically, the determination of their catalytic activity would rather be a point of differentiation between certain members, *i. e.,* eclampsia and nephritis, than a point of resemblance.

CONCLUSIONS.

1. In work along this line it is most important to have a simple method of determining the catalytic activity of tissues. The method must be such that several observations can be made during a single experiment, so that a better idea can be obtained of the velocity of the reaction at various intervals.

2. The catalytic activity of human tissues varies greatly in diseases.

A. *Nephritis.*—The kidney always shows the most marked reduction although the other tissues examined, blood, lung, liver, spleen, likewise show decrease in their power of decomposing hydrogen peroxide. This reduction varies directly with the severity of the pathological lesion in the kidney and the clinical symptoms. The urine also in cases of nephritis shows a much greater inhibiting power than normal urine. This may be accounted for by the reaction of the urine, and subsequent work must prove whether or not the kidney takes any more active part in nephritis and secretes into the blood and urine a substance which manifests itself by a reduction in the catalytic activity.

B. The catalytic activity of the blood in the two cases of eclampsia which we have studied was not reduced. This is the most important fact we have so far obtained, if it can be substantiated, since it can furnish us with a ready ante-mortem means of differentiating eclampsia and nephritis.

C. *Pneumonia.*—The lung in the stage of *red hepatization* has an increased catalytic activity. This increase varies directly with the number of intact red blood cells in the exudate, and in the engorged capillaries. Strength is given to this conclusion by the

fact, that on the one hand, there is no increased activity in *gray hepatization,* while on the other hand, there is an enormously increased activity in the fresh *hemorrhagic infarct.*

D. *Tuberculosis.*—The decreased activity of the lung in tuberculosis is probably due, for the most part, to the lack of blood in the diseased area, while the lowered activity which is present in the other organs is to be explained by the anæmia and emaciation which accompanies the process. Whether there is a specific catalytic inhibiting substance generated by the process, has not been determined.

E. There was no reduction of the catalytic activity in the cases of diabetes mellitus and jaundice studied.

F. In the one case of asphyxiation by illuminating gas, there was decided decrease in the catalytic activity of the blood.

G. The tissues in the one case of congenital syphilis showed a marked lowering of the catalytic activity.

3. There is a slight decrease in the catalytic activity of the tissues due to post-mortem change, but this is so slight that it is hardly to be taken into consideration in the interpretation of the results obtained.

4. There is no marked change in the catalytic activity due to age.

In concluding, we wish to thank Dr. A. S. Loevenhart, at whose suggestion this enzyme was studied, for the unfailing interest with which he has followed the progress of this work, and for the many valuable suggestions he has given us.

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ON THE EFFECT OF COMPLETE ANEMIA OF THE CENTRAL NERVOUS SYSTEM IN DOGS RESUSCITATED AFTER RELATIVE DEATH.[1]

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Experimental studies on the effect of anemia on the brain and spinal cord are recorded as early as 1667, and are numerous, particularly of late years. Analyses of the various results obtained are given by Hayem and Barrier[2] up to 1887, and by Hill[3] and by Battelli[4] up to 1900. Reference to preceding work is also made by Prus[5] and by D'Halluin.[6] No further review, therefore, seems necessary. The latest and most valuable contributions are those of Stewart and his collaborators, Guthrie, Burns, and Pike,[7] to which, as well as to certain of the results of others, reference will be made later.

The method employed by the majority of investigators has been occlusion, either of the cerebral vessels or of the aorta at various levels. The objection to this method, which is frankly acknowl-

[1] The present article is the sequel of that on the Technique of Resuscitation, published in this Journal, Volume viii, 1906, but unfortunately circumstances incident to the severance of our former relations before its completion prevented an earlier presentation. Received for publication June 27, 1908.

[2] Hayem and Barrier, *Arch. de physiol. norm. et path.*, 1887, x, Series 3, 1.

[3] Hill, *Philos. Transactions of the Royal Society*, 1900, cxciii, 121.

[4] Battelli, *Compt. rend. de l'Acad. des Sciences*, 1900, cxxx, 800; *Jour. de physiol. et path. gén.*, 1900, ii, 443.

[5] Prus, *Wiener klin. Woch.*, 1900, xiii, 451, 482; *Arch. de méd. exper. et d'anat. path.*, 1901, xiii, 352.

[6] D'Halluim, *Compt. rend. de la Soc. de Biologie*, 1905, II, 370; *Presse méd.*, 1904, xii, 345; Thesis, Lille.

[7] Stewart, Guthrie, Burns, and Pike, *Jour. of Exper. Med.*, 1906, viii, 289; Guthrie, Pike, and Stewart, *Am. Jour. of Physiol.*, 1906, xvii, 344; Stewart and Pike, *ibid.*, 1907, xix, 328, xx, 61; Stewart, *ibid.*, 1907, xx, 407; Pike, Guthrie, and Stewart, *ibid.*, 1907, xxi, 359; Stewart, Guthrie, Burns, and Pike, *Jour. of Exper. Med.*, 1908, x, 371. (The last article contains a résumé of the literature on resuscitation.)

edged, is that the factor of collateral circulation cannot be entirely eliminated, even in the most favorable animal, the cat. Further, either the brain or the spinal cord has been separately investigated, which must make a vast difference, not from the side of determining the relative viability of the various centers, but in fixing the limit of anemia admitting of a complete recovery of the animal. Stewart[8] says, "Division of the cord in the upper dorsal region (III to VI dorsal vertebræ), if done before fairly complete recovery of the cerebral centers, is followed by collapse, dilatation of the pupil, cessation of respiration, cardiac failure and death. The integrity of the spinal centers is necessary for the resuscitation of the cerebral centers."

Other methods of investigation employed, such as artificial circulation through the decapitated head, and the introduction of emboli, preclude the possibility of recovery studies. Within the last decade, with the more or less successful attempts at resuscitation of the heart which has been made to stop beating, contributions to the subject of anemia of the whole central nervous system incidental to this state of relative death and its bearing on the possibility of general systemic recovery have been made by several observers, notably Prus and Battelli. Given a successful method for restoring to its normal functional activity a heart which is quiescent but viable, while not only interesting as an isolated phenomenon, but also promising fruitfulness in adding to our store of information, its main value is not absolute but dependent upon the power of resisting the malign effects of the cessation of the circulation possessed by the other organs and tissues. That the nervous system naturally comes first in the consideration of such a relation needs no discussion.

This study of brain anemia was the sequence of our work on the resuscitation of animals killed by anesthetics and asphyxia,[9] which may be briefly summarized as follows for the purpose of this paper. By means of a centripetal arterial infusion of salt solution under sufficient pressure, together with the injection of one to two cubic centimeters of 1–1,000 adrenalin chloride in mass

[8] Stewart, Guthrie, Burns, and Pike, *Jour. of Exper. Med.*, 1906, viii, 389.
[9] Crile and Dolley, *Jour. of Exper. Med.*, 1906, viii, 713.

dose early and along with the infusion, and the simultaneous employment of vigorous artificial respiration and gentle but firm cardiac massage through the unopened thorax, a heart which has ceased to beat may be excited to resume beating within certain limits. Up to five minutes of total cessation of function these efforts are uniformly and readily successful, provided that the full technique has been used; up to ten minutes, there is an occasional failure, but after that the chances of success become progressively less, and our limit was thirty-five minutes in puppies (three cases). While it is true that this method in our hands was not efficacious in resuscitating a quiescent heart after a longer interval, which result has been attained by prolonged direct massage (*e. g.*, after one hour, by Prus), nevertheless, it is uniformly successful within the limits which are compatible with viability of nervous tissue, both according to our own results and the consensus of opinion of later writers. The method is necessarily self-limited, for in the case of a heart which is losing its irritability owing to lapse of time, dilatation occurs from the infusion before the requisite amount of stimulation is given, and as maintenance of pressure in the coronary arteries is the most essential factor, the infusion must be continuous. Indirect massage of the heart by compression of the chest, while generally viewed as insufficient, is a subsidiary factor, and has proved satisfactory, even in dogs with rigid chest walls. In periods under five minutes, hardly any is necessary. In no case in our experience has clotting in the heart been an obstacle without concomitant and unnecessary cardiac trauma.

Our purpose being primarily to determine the period of anemia the central nervous system could stand with subsequent recovery, the method offers the advantage that no operative procedure is necessary except the small incision for inserting the infusion cannula. Our results are based, first on a series of thirty unselected dogs, resuscitated after various times, in all but five of which the subsequent course of events was not disturbed. These five were killed after different times for the purpose of histologic examination. Secondly, the series of sixty dogs, previously reported in the paper on resuscitation, was drawn upon for data pertaining to this work. In the latter experiments blood-pressure and respiratory tracings were made.

TECHNIQUE.

For the recovery experiments, with one exception, the dogs were killed by chloroform. While open to objection on account of the paralyzing effect on the nervous system, and the non-elimination until after the resuscitation, this method was adopted in imitation of the condition most likely to afford opportunity for resuscitative measures within the time limits of successful application, *i. e.,* respiratory and cardiac failure in the course of surgical operations. All procedures were done with the customary aseptic precautions. Ether was used for preparation, and the infusion cannula was inserted in the axillary artery, thus saving the cerebral vessels. In the majority of cases ten minutes under ether sufficed, in a few fifteen were necessary, and exceptionally, somewhat longer. As little ether as possible was given and the dog was allowed to come well out of its influence before starting the chloroform. This was usually administered from a well-ventilated cone, though sometimes through the throat tube. The latter method was employed when this tube for future artificial respiration was applied before respiratory failure. The throat tube, which was tried to obviate the necessity of an operation for a tracheal cannula, proved most satisfactory, and would doubtless be equally efficacious for the human subject. It is bent to conform to the oral cavity, with one end somewhat pointed and with a small flange one inch therefrom to catch behind the vocal cords.

In order to estimate in some measure the effect of the amount of anesthesia on the subsequent course of the dogs, two experiments were made of anesthetizing the dog in the usual way to the point of respiratory failure and resuscitating it by artificial respiration without permitting cessation of the circulation. The first dog showed full return to intelligence in sixteen minutes. The second one took ether like an alcoholic, and it was forced for ten minutes before struggling ceased. However, in twenty minutes after recurrence of the respiration the animal walked about and showed return of functions, though extremely groggy.

The period of total anemia was estimated to start from the moment when the first heart sound ceased to be audible with the stethoscope, this sound sometimes persisting for several minutes

after the failure of blood-pressure and the disappearance of the carotid or femoral pulse and the second sound. While this is only the statement of the well-known fact, attention is called to it because very possibly in some experiments it makes our stated period of anemia shorter than it actually was, with a circulation too feeble to reach the brain. In some cases, with the heart sounds becoming fainter and fainter, it was impossible to record the actual moment of failure. In these a leeway of at least one-half minute was allowed from the last distinct sound to the recorded cessation. From the time of starting the chloroform to respiratory failure there was an average of two and three-fourths minutes, with a minimum of fifteen seconds and a maximum of seven and one-twelfth minutes (Table I). The stoppage of respiration and the final failure of the heart were synchronous in five cases, from which the intervening time varied up to six and three-fourths minutes, with an average of one minute and fifty-seven seconds. From a study of the tracings of the first series it was found that the blood-pressure was at the base line during an average time greater than the latter half of this period. It is probable, therefore, that during this period the cerebral circulation was reduced nearly to the vanishing point, and while a small amount of blood goes a long way in the brain, Leonard Hill saying, "It is obvious that the cortex can be kept from death for hours by the merest dribble of blood," this marked anemia of several minutes duration had an effect which was apparent in that the cases with prolonged partial anemia did not recover to the same degree as the average dog subjected to total anemia of the same duration. It is worthy of note that in the dog of maximum recovery, which was one and one-third minutes above the time of the second best result, only twenty-two seconds intervened between respiratory and cardiac failure.

In five out of the twenty-nine animals killed by chloroform, there was a brief spontaneous recurrence of the heart sounds, occurring from twenty seconds to one and one-half minutes after they had entirely ceased, and accompanied in two by two or three faint respiratory efforts. In only one was the carotid pulse palpable. As their total time cannot be exactly classified, both their absolute and practical times will be given.

The time spent in resuscitation is included in the period of total anemia. While it seems reasonable to suppose that a centripetal arterial infusion of salt solution aided by indirect massage of the heart would hardly reach the brain to any extent during a period of administration of from one to three minutes, and, further, from the consideration that if it did, there would be very little blood in the salt solution, the question was put to the test of experiment. In a dog dead for twelve minutes, a solution of methylene blue was infused into the axillary artery, and the usual procedures, with the exception of adrenalin injection, were carried out for double the average time. No indication of its having reached even the bulbar centers was found. This is in contradistinction to direct massage, which effects a veritable artificial circulation, according to D'Halluin, sufficient to reanimate and maintain bulbar activity. Study of the tables of Prus shows that by direct massage respiration returned in twenty-nine out of thirty-five experiments in one series, though there was no return of effective heart beat, and in seven cases, reflexes and muscular movements reappeared.

The definition of the end of the period of total cessation of the circulation was, however, sharp, the resumption of function on the part of the heart being abrupt, visible as well as palpable. After a few initial sounds blood pressure rose rapidly, often within ten seconds, to as much as 200 mm. Hg or over, due to the adrenalin effect.

RESULTS IN RECOVERY (Tables I and II, p. 807).

Permanent and complete recovery was obtained after five minutes, six minutes,[10] six minutes and ten seconds, six minutes and fifteen seconds,[11] and seven minutes and thirty seconds of total cessation of the circulation. That is, one dog out of twelve with total cessation of circulation between the periods of seven minutes and eight and one-half minutes recovered, whereas only one out of seven between the periods of five minutes and six and one-half

[10] Recurrence of heart sounds interjected for thirty seconds; practical time six minutes and thirty seconds.

[11] Recurrence of heart sounds for one minute and twenty seconds; practical time six minutes and thirty-five seconds.

minutes died apparently as a direct result of the anemia. Complete recovery was presumptive in another dog after seven minutes and thirty seconds. This was an early animal of the first series when technique was the object in view; it was killed on the second day on account of suppuration around the tracheal cannula. He was walking around in good general condition, though no specific examination was made. One dog of the second series, after five minutes and thirty seconds, was killed after twenty-four hours for the purpose of histological examination. Compared with the others of the same degree his condition assured a probable recovery. The asphyxiated dog, five minutes and forty-five seconds in time, was killed on the fourth day on account of a gangrenous inflammation of the axillary wound. A second animal of the first series, seven minutes in time, was killed on the third day since he appeared moribund. He was found to have thrombosis of both sides of the heart, with clots extending into the arteries—a sequel of violent massage. Excluding these accidents, the recovery of the last two animals would have been probable, since no dog that died lived longer than thirty-six hours, in all but two death occurring under twenty-four. Our experience shows no intermediate condition of fatal outcome delayed for several days uncomplicated by accidental organic lesion, in other words, no slow decline to death. The demarcation between recovery and death is a sharp one. The crisis in practically all the experiments was reached in from twelve to twenty-four hours. Then death quickly ensued or distinct improvement of nervous functions shortly began, continuing more or less rapidly until complete restoration, though the convalescent period lasted in two dogs four and six weeks respectively.

From his studies on the effect of different degrees of anemia by means of occlusions, Leonard Hill says: "The degree of anemia required to produce dementia is separated by the narrowest line from that which produces coma and death of the respiratory center. There are either no symptoms or death in a few hours." Our results accord with this statement. Up to a certain point, not to be exactly limited, but roughly six minutes, the after-effects are not marked and the second, third, or fourth day brings com-

plete recovery. For example, one dog (Experiment 10), after four minutes and ten seconds, showed entire return of intelligence under one hour, evinced partly in well-defined efforts to escape from the laboratory, while another after six minutes and ten seconds (Experiment 29) showed general return of function within twenty-four hours. Beyond the six-minute limit, however, there is a great deal of after-effect, increasing out of all proportion to the increase in the duration of the period of anemia, reaching as well in the dogs which finally recover a temporary state in which the animal is little more than a cardio-respiratory mechanism, and beyond this limit recovery is altogether uncertain, but if the stage of depression is tided over, recovery has been eventually complete in our experiments, though the narrowness of the escape is indicated by the degeneration of a certain number of neurons in the recovered dogs whose brains were studied by the Marchi method. This does not exclude the possibility of a partial recovery in the sense of a permanent localized after-effect, such as the paralysis of one fore leg described by Stewart[12] in two animals which, however, were under observation only seven and nine days, respectively. In our dog with recovery after anemia of maximum duration, it was the pyramidal fasciculus in which the degeneration predominated. The distinction here has reference to the ability of the whole organism to maintain life at all. The viability of the vital centers, as well as other centers, is considerably above that of the brain as a whole, as the results in recovery prove, and the immediate outcome must depend on the maintenance of the inter-relation and association of all centers, cortical and subcortical. Stewart[13] says that, when exposed to adverse influences, the synapse proves the weak link in the nervous chain.

In general the following sequence of return of the various functions and reflexes was exhibited: respiration, vasomotor control, corneal reflex and knee jerk (tendon reflexes in general), winking, cutaneous reflexes, partial or complete contraction of pupils and light reflex. This order was subject to considerable variation, which will be considered under the special discussion of func-

[12] Stewart, Guthrie, Burns and Pike, *Jour. of Exper. Med.*, 1906, viii, 289.
[13] Stewart and Pike, *Amer. Jour. of Physiol.*, 1907, xix, 3, 328.

tions and reflexes. Hypertonicity of the voluntary musculature immediately succeeded recovery of a normal tone and was manifested by exaggeration of the knee-jerks if not by a more or less widespread spastic condition. It always followed rapidly the reappearance of the knee-jerk. Reflex muscular movements, secondary to skin or tendon stimulation, always preceded those of spontaneous origin. Spontaneous incoördinate movements appeared sometimes before, sometimes after the light reflex, but afterward only when the light reflex returned relatively early. Incoordinate movements followed quickly in the dogs with anemia of short duration, so that perhaps the incoördinate movements did not appear, but the resumption of muscular activity was sudden, the dog starting up as though suddenly aroused from sleep. Succeeding the coördinate movements were what may be classed as purposeful movements, involving all the muscles of locomotion and being attempts to turn over, to arise, or to crawl forward. Usually after the appearance of coördinate movements, less frequently about the same time, visual and auditory reactions were obtained. The auditory was always the more definite, and usually returned first. It is the combination of the return of the special senses with purposeful muscle movements, all together or in part, that is termed consciousness, though it has been usually very dim and uncertain. In many of the dogs which succumbed after some hours there was more than a mere reflex revival, there being some manifestation of the higher faculties in addition.

The course of events following the resuscitation may be summarized as follows: A state of hyperexcitability follows reanimation, reaching its maximum in one to three hours, when retrogression begins. This second stage is characterized by the onset of uncontrolled muscular movements, either coördinate or convulsive, lasts a longer time, and gradually passes into the third stage of depression and paralysis, in which the reflexes are more or less involved. The dogs which recovered from this never exhibited as much mentality nor such an activity of reflexes on the second day as for a short time after the resuscitation. Consequently, it is our opinion that the extent of temporary recovery in the dogs which died was merely a manifestation of the first stage of hyperexcitability.

The average picture toward the end of the first stage, for a recovery dog, is one of an animal in a very stuporous condition, for the most part lying quietly, with an accelerated pulse and much quickened respiration, expiration being prolonged and more labored, with normal conjunctival reflexes, with cutaneous reflexes constantly present in the paw stimulated but less so in the homolateral fore-limb, and least so in the contralateral hind-limb, with exaggerated tendon reflexes, and with pupil reflexes, if present, uneven and tardy. There is a general spastic condition of the muscles, the legs being commonly held in extension. On loud sounds in close proximity, the head may be raised, the eyelids opened with dilating pupils, and the ears pricked up, the attitude being simply one of attention without localization or indication of ideation. To a flash of light, the head may be withdrawn, but usually there is only a lid reflex. Especially on disturbance but sometimes without it, the animal arouses, barks, looks around, and exhibits coördinate and even purposeful and propulsive movements of the legs and body, attempting to rise or to crawl, usually unsuccessfully, but at times succeeding in getting to the standing posture or in a short progression, lasting a few moments, depending on the extent of the paralysis, and then falling at once into stupor.

The second stage, of retrogression, was a constant phenomenon in all the dogs but was much less marked after the shorter times The animal becomes progressively more comatose, the spastic condition, which had largely disappeared with the reanimation, returns, visual and auditory reaction disappear, and the skin reflexes become inconstant. Muscular movements, however, are kept up, but are less coördinate and more spasmodic. This seems to depend on the duration of the anemia, convulsive movements being little marked after the shorter times. On the other hand, the dog with recovery from anemia of maximum duration had definite tono-clonic convulsions. When such convulsive movements are well marked, after a period of hours the animal passes into a deep coma, with a general condition from which recovery hardly seems possible. This lasts well into the night, but the second morning finds a distinct improvement.

Up to this point, the course of events in the animals which

succumbed, embracing all but one with anemia over the seven-minute period, while limited according to the extent of the recovery, was similar to that in the dogs which did eventually recover. In the usual sequence, a few did not attain a light reflex and the majority came back so far as to exhibit coördinate movements and auditory reaction, but only in two was there apparently dim consciousness. Reanimation of the higher faculties was much more transient so far as it occurred, and the animal passed more quickly into coma from which it was impossible to arouse. Irritability of the muscular control was much more marked, with a few exceptions, periods of violent convulsive movements alternating with periods of quiet and stupor. While these muscular movements were partly clonic and tono-clonic in character, commonly an element of coördination was maintained, and they were distinctly propulsive and progressive, though violent and uncontrolled.

Of the coördinate movements, the running motion of the legs such as is frequently seen in the early stage of anesthesia was the best example, and was performed with extreme rapidity, the dog lying partly upon its back with its legs waving rhythmically in the air. Sometimes only the fore-legs were involved, but usually the movement was general. If the dog was not so completely paralyzed, this resulted in a crab-like progression about the room. The strictly convulsive movements were very complex, clonic and tonic types being mingled, with a violent threshing about. In several instances there was some opisthotonus. The slightest disturbance provoked the movements characteristic for the animal.

The quiet periods in the early stages were of short duration, but the interval was distinct. In the later stages, they became more and more prolonged. Finally, as if worn out, quiet would ensue, the animal being perfectly limp, tendon and skin reflexes first disappearing, next the eye reflexes, leaving only the cardio-respiratory functions, with respiratory failure not far off.

To show the slow return of faculties and the paralyzing effect of the maximum period of anemia from which recovery was made (seven minutes and thirty seconds in Experiment 27), the subsequent course of the dog in this experiment will be given in some detail. On the second day she was awake but paid no attention to

her surroundings. To loud sounds there was only an occasional response and blindness appeared absolute. The hind legs were entirely paralyzed, but when the hind quarters were supported she was able to walk on the fore-legs, though the progression was cross-legged or spraddling. Sensation was much more deficient than motion, no attention being paid to prolonged immersion of any foot in cold water. A strong bull-dog clamp on a hind paw was not noticed beyond restlessness, though on a fore-paw it was vaguely localized after several minutes, but only to the extent of a reflex from the leg itself, and there was no coördinate attempt at removal. Food was not recognized when placed in the mouth and was held there passively. No notice was taken of tobacco smoke or ether fumes. The third day she walked about, though unsteadily and with a decided stringhalt gait of the hind-legs. Water was recognized after standing in it for several minutes. The legs gave way when she jumped off a chair. Hearing was very acute but vision appeared to go no farther than mere perception of light. Localization of sensation by the clamp test was improved to the extent of biting rather aimlessly and very incoördinately at the foot affected, very frequently the wrong leg being attacked if in juxtaposition. It was not until the seventh day that the clamp was recognized, grasped and pulled off. On the third day also the feet were withdrawn from cold water, but the sense of position was not evident till the fourth day, tested by resting the feet on boards at different levels. Only maximum differences of level were then recognized. While the animal appeared hungry, constantly licking her chops, food was not recognized until the fifth day. It was after a week that tobacco smoke and ether fumes were noticed.

While in a week's time the return of all faculties with the exception of vision was indicated to a greater or less degree, the animal was by no means normal but exhibited great hebetude in all respects. She was very lethargic, would not run nor play, and response to any stimulus was delayed and feeble. How much of this was due to the blindness, it was impossible to say, but certainly not all. For two weeks there was hardly perceptible improvement in vision. She paid no attention to attempts at testing it and when she moved about it was slowly, feeling her way, though about

the end of this time she would notice moving objects within a range of a foot or two, with distinctly more accuracy when they were held above the level of her eyes. From this time improvement steadily continued and in four weeks recovery of vision was complete. With recovery of vision the condition of dementia entirely disappeared, and not till then did the psychical faculties of fear, pleasure, and memory become normally evident.

A summary of the extent of temporary reanimation in the dogs which succumbed at such a time as to permit sufficiently exact observation is arranged in the sequence before given as follows: Of seven dogs with anemia ranging from five to seven minutes, one died after twenty-four hours. If he returned to consciousness at all, the return was exceptionally tardy as compared with that of the others with the same period of anemia. Of those dying after periods of seven to eight minutes, two revived as far as spontaneous coordinate muscular movements, without visual or auditory reaction or return to consciousness, while one failed of vision. Of five after eight-minute to nine-minute periods, one did not show even the light reflex and much less did it come out of coma; two recovered to the extent of muscular movements, a fourth added auditory reaction, while in only one was there a suggestion of a return to consciousness. Of three, after nine-minute to ten-minute periods, one went as far as apparent consciousness, one failed at vision, and the third showed nothing beyond the light reflex. One dog at twelve minutes and ten seconds gave only a reflex revival, and in the dog of the recovery series with the longest period of anemia only coördinate muscle movements appeared in addition.

SPECIAL PHENOMENA FOLLOWING RESUSCITATION.

Respiration.—(Table III.) Respiration has recurred in every animal in which the circulation was restored and maintained sufficiently long. The maximum case, thirty-two minutes of total anemia, gave respiratory recovery twenty-one minutes after circulatory to the extent of three faint gasps, but cardiac failure immediately ensued. Comparison of the time of restoration of respiration in our experiments with that recorded by several other observers gives an interesting result, *i. e.,* a much more rapid re-

covery. For our experiments, the average time for restoration for animals between the periods of three and eight minutes of total anemia was three minutes and fourteen seconds. The average time after occlusion of vessels in Stewart's cats and dogs between the same periods was seven minutes and forty-one seconds; for Prus' chloroform series with periods between three and five and one-half minutes was seven minutes and nineteen seconds (with frequent return of respiration before efficient heart beat). Battelli's successful chloroform cases of the same time are too few for comparison, but in general both these and the electrocuted dogs concord with the above results. Hill[14] says that a certain arterial pressure is necessary to invoke respiration. This comparison points to the increased effectiveness of the higher pressure due to adrenalin.

The first gasp was distinct and fairly strong. After a few gasps, in the majority of the animals, inspiration came to exhibit a triple character, with the inspiratory-expiratory ratio three to one, such as occurs in sobbing. This lasted for several minutes. Gradually the rate increased and the rhythm became regular. A sudden assumption of the normal type of breathing sometimes happened in the dogs with short time of anemia and was always associated with simultaneous recovery of the eye reflexes. A rapid increase of rate was the rule in all cases, as much as 100 per minute being recorded, but usually the high rate did not continue for long. For example, it was 72 per minute sixteen minutes after resuscitation in the dog which recovered after anemia of seven and one-half minutes and fell to 36 in about twenty minutes. Afterward it was subject to frequent changes, and not till the third day was a normal rate established in some cases. When the rate slowed, a prolonged and labored expiration was characteristic. The changes of rate in the initial respirations will be best illustrated by reference to the protocols of two experiments.

EXPERIMENT 27.—Hound puppy, about eight months old. Seven and one half minutes total cessation of the circulation.

11.45.30. Return of pulse suddenly appearing in the tongue is first noticed.

11.47.30. First respiration. The first four respirations occur at thirty second intervals (4 per minute) and then for about three minutes at seven second

[14] Hill, The Physiology and Pathology of the Cerebral Circulation, London, 1896, p. 132.

intervals, the inspiratory portion comprising three distinct efforts in all. Fibrillary contractions of the tongue occur after beginning of respiration.

11.55.00. Lachrymal secretion begins. The right pupil is distinctly contracted.

11.58.00. Respirations rather suddenly assume normal character and rhythm with rate of 24.

12.00.00. Respiratory rate has jumped to 48. (Noted further above.)

EXPERIMENT 30. One year old puppy. Twelve minutes and ten seconds total cessation of circulation.

11.30.15. Heart beats, recording from first observation of the pulse in the axillary cannula. Though the infusion tube is partly clamped by the finger, pressure is sufficient to drive the blood into the infusion bottle at a height of five feet.

11.32.25. First respiration occurs. The movement and those following for several minutes are deep and gasping. For four minutes the rate is very irregular, at intervals varying from five seconds to one minute. Artificial respiration is kept up continuously during this time but is stopped as soon as spontaneous respirations become more regular.

11.37.00. Respirations now occur at three to five second intervals.

11.38.00. Respiratory rhythm is more steady (20 per minute) and movements more shallow.

11.44.22. Respirations, 28 per minute.

12.00.00. Respirations, 32 per minute, are somewhat more irregular and spasmodic. Expiration is distinctly labored.

12.13.00. Respirations, 40.

12.40.00. Resprations, 32. The dog is evidently failing, and about one hour later is found dead.

Blood Pressure.—(Table III.) The use of adrenalin complicates the study of the blood pressure changes. In a successful resuscitation, as already noted, the blood pressure rose rapidly, often within ten seconds, usually to a height of 200 mm. of Hg and in one case, 250 mm. This level was maintained from two to five minutes in all but the dog with 250 mm. of pressure, and then it began to fall as the effect of adrenalin was dissipated. From ten to forty minutes elapsed before the lowest level was reached. Depending on the extent of the vasomotor reactivation, either a tendency to rise was immediately exhibited or the low level persisted for from ten to twenty minutes, in the latter case with a subsequent rise. In two animals, after total anemic periods of seven minutes and thirty seconds and eight minutes and thirty seconds, one of which at least may be credited with presumptive recovery, this second level was maintained until the animals were killed—in twenty-four hours and in five and two-thirds hours respectively—but in the others, all

with anemia of longer duration, it was only a temporary reanimation, and along with the reflexes, steadily declined to death.

On account of the effect of adrenalin overlapping the return of vasomotor activity, particularly after the shorter periods of anemia, the relative time of the reactivation of the center could not be absolutely determined. Stimulation of the sciatic nerve did not cause the usual rise of pressure until the secondary rise before mentioned had begun, and respirations were well established before this rise (see Table IV). In fact, with the exception of one experiment in which respiratory recovery was synchronous with the first successful stimulation test obtained shortly before a manifest rise, respiration in all experiments had returned well before the end of the first fall. In one experiment of the second series, as little adrenalin as possible was used. The anemia lasted five and one-half minutes (two-minute recurrence of heart), and respiration began in two minutes after restoration, while reaction to sciatic stimulation was not obtained for four minutes. Synchronously with it, respiration rather suddenly assumed a more normal type. It appears, therefore, that the return of the vasomotor center is nearly synchronous with respiration after the shorter times of anemia, but is more delayed after the longer times. In the puppies after thirty-five minutes there was apparently no vasomotor reactivation.

Reflexes.—(Table IV.) While varying considerably in the time of their recurrence, after the same amount of anemia, the corneal reflex and spontaneous winking returned in all but the three experiments which did not proceed sufficiently long. Up to eight minutes the light reflex reappeared constantly, though it was the least uniform in time and degree. After eight minutes it was inconstant. The maximum time after which the corneal reflex reappeared was twenty-four minutes (maximum resuscitation in adult dogs), and for the light reflex the maximum time was fourteen and one-half minutes.

The knee-jerk varied the least in its range. It was also noted in the maximum resuscitation. Not infrequently there was a difference in the time of recurrence of the bilateral reflexes, in two cases one corneal reappearing three minutes before the other, though for the knee-jerk no difference over one-half minute was noted.

As to the relative time of the reappearance of reflexes, first place belonged to the knee-jerk more frequently than to the corneal reflex, though they were synchronous four times. The corneal reflex always preceded spontaneous winking while in every case the cutaneous reflexes returned before the light reflex.

Temperature.—While not recorded as a routine, sufficient data have been obtained to indicate that the temperature continues to fall for several hours following the resuscitation. The lowest temperature per rectum was 32.9° C. four hours after anemia of nine and one fourth minutes (Experiment 1), and 33.8° C. was reached in sixteen minutes after thirteen and one-third minutes of anemia (Experiment 5). From this point the temperature gradually rose to a state of hyperpyrexia, which was more marked in the animals which succumbed. In the dog which recovered after the maximum period of anemia the maintained level was reached the second day.

Phonation.—Nine and one-quarter minutes (Experiment 1), was the maximum after which this faculty returned. Actual barking, indeed, occurred in but one other case over seven and one-half minutes (Experiment 14), though there was whining or imperfect attempt at vocalization in three. Phonation usually appeared synchronously with or shortly after the exhibition of spontaneous muscular movements, that is, one-half to one hour after resuscitations following anemia of about seven minutes.

Micturition or Defecation.—Micturition or defecation occurred in the majority of animals during the period of hyperexcitability.

Auditory, Visual, and Olfactory Senses.—As already indicated, the reaction to auditory stimuli was definite and unmistakable during the period of hyperexcitability in the recovered dogs, while to various visual stimuli during the same time, the only response was a lid or pupil reflex, but out of fifteen animals with anemia of duration including and over seven minutes, only six gave even a temporary recovery of hearing. Further, in the animals which recovered, the later effects on vision were much more marked, in general increasing as the limit of recovery was approached. For example, in the dog with recovery after maximum anemia, hearing was reasonably acute on the third day, though vision was not fully restored for three weeks, while in the animal with anemia of six and

one-sixth minutes (Experiment 29), the best example of early visual recovery, hearing was normal on the second day, though a day more was required before the animal ran about without collision with obstacles. The maximum period after which hearing was observed was nine and three-fourths minutes (Experiment 15). The sense of smell came back between hearing and vision, though the test was never definite unless irritating fumes were employed. The first reaction to these was on the third and seventh days, respectively, in dogs with anemia of six and seven and one-half minutes (Experiments 2 and 27).

Phenomena Referable to the Cortex.—Most of the animals which recovered passed through a final stage comparable in many respects to the condition of Goltz's[15] decerebrates; such a period was characterized by dementia and loss of intelligence, the lack of any psychic response to stimuli, and the inability to recognize food and drink. Response to stimulation was purely reflex, or absent if memory of past experiences was involved; for example, meat placed in the mouth was held there passively or in one case forcibly spat out, a flash of light was answered by a lid reflex, and there was indifference to the relative position of the fore-legs. Power to localize stimuli was of gradual acquirement. Restlessness, however, was generally not observed.

That the temporary paralysis was of cortical origin was indicated by the associated exaggeration of the knee-jerks. The motor function did not suffer as much as the sensory, for the paralysis disappeared distinctly before the return of intelligent and normal response to stimulation. The clinical observation that the cortex suffered the most and was the last to recover is supported by the fact that the histological alterations were more marked in the cortex than in the lower centers.

THE HISTOLOGY OF FATAL ANEMIA AND OF ANEMIA WITH RECOVERY.

Nissl's Method.—The neurocytes stained by Nissl's method were studied in ten cases, as follows: after short periods of anemia with rapid general functional recovery, the brain being removed one

[15] Howell, Text-book of Physiology, Philadelphia, 1905; and Schäfer, Text-book of Physiology, Edinburgh, 1898–1900.

hour after resuscitation (Experiments 10 and 12); in one case of most probable eventual recovery, twenty-four hours after the resuscitation (Experiment 8); in one case well beyond the limit of probable recovery, one and one-third hours after the resuscitation (Experiment 9); and in six animals which died (Experiments 1, 5, 7, 14, 15 and 16). In several of the fatal cases the brain was removed immediately after death, and in all the others the material was obtained before or almost before the heart's beat had ceased. In all cases, sections were made from the different parts of the central nervous system from the cord to the cortex. The tissue was fixed in 96 per cent. alcohol, and for cutting fastened to the blocks with celloidin without imbedding. The alterations described are based entirely upon a comparison with three control animals subjected to the same amount of anesthesia, in which the stainable substance was as a whole uniform and well defined. Only the motor stichochrome cells were considered, though frequently the changes were so marked that they could be diagnosed only by their size and relations. In studying the preparations comparatively, the plan was adopted of making differential counts of a fixed number of cells, the changes being classified into slight, moderate and marked, and tabulating them.

The effect of anemia has been previously described by Mott,[16] who used the materials from Hill's experiments on occlusion, and by Sarbo[17] and Marinesco.[18] The present work confirms their observations; briefly, the changes, where slight, consist in a swelling of the tigroid masses, where more marked, in partial or complete disintegration, either into smaller granules or into fine dust-like particles with a diffuse staining of the cell. The periphery of the cell with its processes suffers more.

The results of the comparative study were as follows. The alterations in the same case showed a progressive increase in severity from the cord and medulla to the cortex. The fatal cases uniformly presented the greatest change, not merely chromolytic, but here and there definitely indicative of cell death. Nuclear stains were also employed, and brought out what the Nissl method

[16] Mott, The Croonian Lectures, 1900.
[17] Sarbo, *Neurol. Cent.*, 1894, xix, 664.
[18] Marinesco, *Presse med.*, 1897, v, 41.

had suggested, namely, the frequency of structural alterations in the cells. In the mildest form only a swollen condition of the whole cell was visible. Serious involvement of the nuclei was manifest frequently in their eccentric position or even in their disappearance. With slighter injury, possibly no nuclear membrane appeared, though the nucleolus remained intact. In fact, the nucleolus seemed to be the most resistant element. Other appearances occurring alone or associated with the preceding were an apparent diminution or an irregular massing of the nuclear material and occasional vacuolation. The marked changes were practically confined to the fatal cases. These findings, supported as they are by evidence of degeneration of fibers, place the limits of experimental resuscitation upon an anatomical basis.

Of the two dogs with rapid functional recovery, one (Experiment 12) after three minutes of anemia matched very closely the controls, but in the other, four and one-sixth minutes, the tigroid substance stained poorly. While it is true that for the time that had elapsed the animal had progressed well toward recovery, and the technique may have been at fault, the experiment suggests an explanation for the two deaths (Dog 17 and Dog 23) which occurred under the usual limit. Dog 17 after the same period of anemia died from respiratory failure in fifty-three minutes, after having progressed to eye reflexes, barking and muscular movements, and Dog 23, after six minutes and five seconds of anemia died twenty-four hours later, its reanimation having been comparatively imperfect. In terms of this explanation, the poor resistance in these animals may be expressed by saying that they were handicapped by a preëxisting functional derangement of their nerve cells. Further, among the fatal cases showing parallel changes, was an early death (Experiment 7 with death in forty-four minutes after seven minutes of anemia) which bears out the preceding. On the other hand, that the anemia alone was not responsible for the appearance was indicated by the animal with an anemia certainly sufficient to cause death (Experiment 9); its course was terminated shortly for examination, and therefore it is comparable as to the time after resuscitation with the last experiment but in staining it ranked next to Experiment 12 with

its rapid recovery. A comparison of this same case with the one twenty-four hours after the resuscitation (Experiment 8) gave differences in favor of the former, that is, after a fatal period of anemia examined shortly after resuscitation there was less change than after a shorter period promising recovery, examined at the end of twenty-four hours. This fact points to a progressive effect following an anemia of the character produced and is in accord with the generally expressed views as to the nature of the Nissl substance. For some time after the resuscitation the cells subsisted upon their stored-up energy. While the histological data upon this point are meager, they fit in with the stage of depression following the stage of heightened irritability uniformly observed.

Marchi Method.—(Photographs.) This method was used in four fatal cases (Experiments 16, 23, 24 and 25) and in two of the recoveries (Experiments 22 and 27). Preparations were made from the optic tract and the spinal cord and medulla and, in addition, from the pons, cerebral peduncles, and internal capsule in the dog with recovery after maximum anemia. This dog gave the most interesting findings. In the cord, with a very few blackened fibers scattered throughout the white matter, the degeneration was localized in the lateral pyramidal fasciculi and in the proper lateral fasciculi of Flechsig. This localization was more marked on the left side, and while only a relatively small proportion of the fibers were involved, it was sufficient to mark out these tracts. Of the pyramidal fasciculus, on the left side nearly one hundred, and on the right side between thirty and forty fibers were affected. In the higher sections, this fasciculus was readily traceable to the cortex by the presence of this degeneration. It is, therefore, apparent that in this animal a small proportion of the psycho-motor cells entirely succumbed to seven and one-half minutes of total anemia. Failure of the cells to recover was escaped by a narrow margin, though the dog, which was killed after one month, gave no evidence of disability. A similar finding is recorded by Mott in one of Hill's monkeys after ligation of both carotids and one vertebral; in ten days the animal had returned to a normal condition, but showed the degeneration of about sixty fibers on the side opposite the ligated vertebral artery.

The other dog which recovered also gave a picture of degeneration, but of a different character. While the actual number of fibers involved was greater, not only was there no localization but there was an early stage of degeneration with droplets of varying size, scattered in longitudinally cut spinal nerves at intervals along the course of the fibers affected. This animal, which was killed in six days was, at the time of death, partially blind and deaf, and though it could stand, it was too paralyzed to maintain the upright posture or to walk. The question arises as to whether it would have eventually recovered. Judging from the other animals which, with final recovery, passed somewhat more rapidly through a similar condition, and from the fact that there was a noticeable improvement from day to day in the animal just mentioned, general recovery, with a complete destruction of a few neurons, is probable.

An early degeneration similar to that mentioned occurred in the fatal cases, though the number of fibers involved was considerably less. None of these animals had lived over thirty-six hours at the outside. On account of the shortness of the time which had elapsed, this appearance was unexpected and will be further investigated. However, it corresponds with the organic changes in the cell-bodies which occurred during the same time.

COMPARISON WITH THE RESULTS OF OTHERS.

Mayer[19] concludes that from ten to fifteen minutes is the limit for general resuscitation, though respiration and the vaso-constrictor activity may recover after that time. Stewart,[20] with complete recoveries after occlusion of cerebral vessels for five, six, eight, nine and five-fifths, and sixteen and one-half minutes, agrees with Mayer's conclusion. Hayem[21] says that in general, brain functions are not recovered after from ten to eleven minutes. The conclusions of Batelli and of Prus are the only ones drawn from observation of animals resuscitated from apparent death. None of Battelli's dogs survived, and he ascribes this fact to the severity of the operation on the thorax necessary for his resuscitative measures of electrization and heart massage; he says that, aggravated by the violent

[19] Mayer, *Med. Cent.*, 1878, xvi, 579, 594.

[20] Stewart, Guthrie, Burns and Pike, *Jour. of Exper. Med.*, 1906, viii, 298.

[21] Hayem, *Archiv de physiol. norm. et path.*, 1888, i, Series 4, 103.

respiratory efforts, the animal passes into coma and dies. However, from the extent of reanimation exhibited, he concludes that the functions of the central nervous system may be reëstablished after ten minutes of total anemia, but not constantly after fifteen, and the maximum limit is twenty minutes. Prus does not fix a definite limit. Stewart suggests that in such prolonged periods of one and two hours as those of Prus, the auricles must have kept up a slow, but in some degree efficient, movement of the blood through the brain. On account of the injury from opening the thorax, Prus attempted recovery in only thirteen out of fifty-two dogs reanimated, the others being killed after a short time. Two of these survived. One was killed by asphyxiation, and the period of total anemia was six minutes, estimating from the stoppage of the heart to the beginning of direct massage, as considered under "Technique." From the data given, the subsequent course of this animal corresponds closely to our six-minute periods. The other was after a period of four minutes killed by chloroform. The dogs that survived from three to five days were all subjected to anemia of short duration, and of the two cases above six minutes, both ten minutes of total anemia, one died in six and one-half and the other in twenty-four hours, all the deaths being ascribed to infection.

From our experience, it seems justifiable to say that observation of an animal resuscitated from a state of completely suspended animation is very misleading unless carried far enough, and does not permit of conclusions regarding the limit of anemia admitting of recovery. For there quickly ensues a condition of hyperexcitability of reflexes, associated with voluntary movements and with greater or less return of the special senses, even with an apparent return to consciousness; consciousness is transient, but appears in animals which succumb as well as in those which eventually recover. In our experiments this phenomenon was exhibited in dogs subjected to nearly double the amount of anemia from which recovery was made, and in several instances the appearance of reanimation was so decided as to make the prognosis very hopeful. But after a few hours, more or less, the special senses failed, the dog became progressively stuporous, convulsions ensued, then loss

of reflexes, and, finally, respiratory failure. The decline to death indicated definitely a nervous origin, and autopsies on all the fatal cases directly following the resuscitation showed in only one organic lesion to which death could be attributed. This was a dog with anemia of eight and one-half minutes (Experiment 14) dying after between thirty and forty hours, with an early and irregularly disseminated broncho-pneumonia. As long a survival as this was the exception.

By the occlusion method, the general conclusion appears to be that ten to fifteen minutes expresses the limit within which resuscitation is practicable. This conclusion is not unassailable for two reasons: first, the impossibility of absolutely eliminating the factor of collateral circulation, and, second, the brain and cord have been separately investigated. In the latter connection, apart from the inter-relationship of the two, the possible percolation upward of the cerebro-spinal fluid,[22] with the circulation of the cord unimpeded, is worthy of consideration.

As a result of our experiments, with a certainly total anemia and little opportunity for infectious accidents—conditions the most favorable for investigation of the possibility of recovery—it is our opinion that the limit should be reduced one-half. For dogs killed by chloroform, the average duration of anemia from which recovery may be made is between six and seven minutes. The ulterior limit appears to us to be under ten minutes, and any recovery over seven and one-half minutes would be exceptional. The practical importance of the accurate fixation of the limit beyond which recovery is impossible is immense, when applied to a procedure of resuscitation which is at our disposal and may be relied upon in the case of accident.

SUMMARY.

To determine the limits of recovery after a total anemia of the central nervous system, a series of thirty dogs was killed by chloroform and resuscitated after varying times from three to fourteen minutes. Under five minutes the recovery of function was rapid and strikingly free from the after effects which characterized longer periods. Of seven animals between the periods of five and six

[22] Hill, Cerebral Circulation.

and one-half minutes, only one died apparently as a direct result of the anemia, but of twelve between the periods of seven minutes and eight and one-half minutes, only one, after seven and one-half minutes recovered. The remaining dogs all died. Further corroborative data are drawn from the previously published paper on the technique of resuscitation.

Histological examination both of presumptive recoveries and fatal cases was made by ordinary methods and those of Nissl and Marchi. The neurocytes of the fatal cases uniformly presented the greatest change, not merely chromolytic but here and there definitely indicative of cell death. Marchi's method further supported these findings by proving the existence of fiber degeneration. Finally, showing the narrowness of the escape, the animal with best result in recovery, seven and one-half minutes in time, which at the end of four weeks had apparently entirely returned to a normal state, by the Marchi method had a degeneration of a number of fibers localized in the pyramidal fasciculi which were traced from the cord to the cortex, and in Flechsig's fasciculus, as well as a more sparsely scattered degeneration of both ascending and descending fibers elsewhere.

We are indebted to Dr. H. V. Wilson, professor of zoology in the University of North Carolina, for his kindness in taking the micro-photographs.

CONCLUSIONS.

1. In dogs anesthetized by ether for preparation, and killed quickly by chloroform the average limit of total cerebral anemia, estimated from cessation of the heart sounds to return of circulation, which admits of recovery, is between six and seven minutes. The ulterior limit appears to be under ten minutes, hitherto stated as the most conservative figure, and any recovery beyond seven and one half minutes would be exceptional.

2. Further experience with resuscitation of animals killed by anesthesia and asphyxia, embracing numerous unrecorded experiments, as well as those forming the basis of the present article, establishes our former conclusion, that the procedures detailed afforded a reliable method within its limitations, and certainly uniformly successful within the limits compatible with the recovery of the central nervous system.

TABLE I.

Duration of Anemia in Minutes and Results in Recovery. (Series I.)

No. of Experiment.	Time from Beginning of Administration of Chloroform to Respiratory Failure.	Time from Respiratory to Cardiac Failure.	Duration of Recurrence of Inefficient Heart Beats After Apparently Complete Failure.*	Duration of Total Anemia.	Results.
12	4⅚	3⅙	0	3	Killed after 1 hour.
10	4¼	1½	0	4⅙	Killed after 1 hour.
17	—	3	0	4⅙	Died, 53 minutes.
3	1¼	4	0	5	Recovery.
22	2	‡	1½	5¼ (6 7/12)	Recovery.
8	2½	½	2	5½ (7½)	Killed, 24 hours.
26	†	¼	0	5¼	Killed on 4th day; secondary infection.
13	2	6	2	6(8)	Killed after 1 hour.
2	2½	1	½	6(6½)	Recovery.
23	2⅔	1¾	0	6 1/12	Died, 24 hours.
29	⅚	‡	0	6⅙	Recovery.
25	1½	4½	0	7	Died after 30–40 hours.
7	—	3¼	0	7	Died, 34 minutes.
11	7 1/12	⅙	0	7½	Died, 15–20 hours.
6	3	3	0	7½	Died, 12–20 hours.
27	2 7/12	22	0	7½	Recovery.
20	2⅓	1⅔	0	8	Died, 10–20 hours.
28	3⅓	1⅙	0	8⅙	Died, 12–20 hours.
18	—	2	0	8¼	Died, 63 minutes.
4	4	1	⅙	8⅓ (8½	Died, about 12 hours.
19	2¾	1⅚	0	8 5/12	Died, about 20 hours.
14	2	1	0	8½	Died, 30–40 hours; disseminated broncho-pneumonia.
24	4½	‡	0	8½	Died, 23 hours.
1	—	6¾	0	9¼	Died, 11 hours.
9	2	2⅔	0	9⅔	Killed, 1 hour.
15	3½	½	0	9¾	Died, about 18 hours.
16	—	—	0	10	Died, 10–18 hours.
30	1 7/12	‡	0	12⅙	Died, about 2½ hours.
5	—	⅙	0	13⅓	Died, 24 hours.
21	2	‡	0	14	Died, 40 minutes.

* As noted on page 786 this column refers to the spontaneous recurrence of the heart sounds in five cases from one-third to one and a half minutes after they had entirely ceased. As the extent of the circulatory recovery could not be exactly estimated, though usually it was not sufficient to produce a palpable pulse, in the next column the duration of the total period without any evidence of cardiac activity is given first, while the figures in parenthesis include the partial recovery.

† Asphyxiated dog.

‡ Synchronous.

TABLE II.

Results in Recovery of Series I.

(In other successful experiments the animals were killed after shorter interval.)*

Number of Experiment.	Total Duration of Anemia.*.	Mode of Death.	Results.
28	7½	Ether.	Killed, 24 hours; resumptive recovery.
19	8½	Asphyxia.	Killed, 6 hours.
31	9½	Chloroform.	Died, 20 hours.
37	9½	Chloroform.	Died, 20 hours.
48	12	Asphyxia.	Died, 3¾ hours.
39	13½	Chloroform.	Died, 15 hours.
41	15	Chloroform.	Died, 15 hours.
30	16⅓	Ether.	Died, 3½ hours.
43	24	Chloroform.	Died, 4½ hours.
49	32	Chloroform.	Died, 27 minutes.

* Includes time spent in resuscitation.

TABLE III.

The Course of Blood-pressure after Resuscitation. (*Series I.*)

(The time is in minutes after restoration of circulation and the pressure in mm. Hg.)

Number of Experiment	Duration of Anemia.*	Time of Respiratory Recovery.	Maximum B. P. after Resuscitation.	Time to Reach First Lowest Level.	Lowest Level of B. P.	Approximate Duration of Lowest Level.	Total Time to Second Rise.	Height of Second Maximum.	Subsequent Course (x)
28	7½	4½	240	10½	80	†	11	160	Maintained for 24 hours.
19	8½	11½	206	14½	130	‡			Maintained for 6 hours.
31	9½	7	210	30	80	§			Removed from table.
37	9½	½	230	40	80	‖	40	90	Removed from table.
48	12	16	220	16	50	10	36	80	Fell after 5 minutes.
39	13½	15½	250	18	80	2	20	120	Fell shortly to 70 mm.; 1 hour.
41	15	8¾	180	28	44	15	43	88	Removed from table.
30	16⅓	15½	144	23	118	3	26	140	Fell after 10 minutes.
43	24	4	110	23	36	5	28	70	Second maximum held 1 hour.
49	32	21	114						Cardiac failure after 27 minutes

* Includes approximate time of the act of resuscitation.
† Immediate rising tendency.
‡ Level steadily maintained.
§ Removed from table in 30 minutes.
‖ In general, a decline to death.

[20] Crile and Dolley, *Jour. of Exper. Med.*, 1906, viii, 713.

TABLE IV.

Giving Time in Minutes of Recurrence of Respiration and Various Reflexes after Restoration of Circulation. (Series I.)

No. of Experiment.	Duration of Anemia.	Respiration.	Corneal Reflex.	Winking.	Light Reflex.	Knee-jerk.	Cutaneous Reflex (First).
12	3	2	4¾	5	38	16	—
10	4⅙	1⅔	10	10½	18½	10	13½
17	4⅙	2⅔	20	25	0	0	0
3	5	⅔	2	*	10¼	2	—
22	5¼	1¾	*	15¾	128	17	23
8	5½	2	13½	16½	18½	12	—
26	5¾	—	12½	—	14½	24½	—
13	6	7 1/13	*	19	†	35	39
2	6	¾	10¾	13	40	8¾	25
23	6 1/13	2	10½	19½	‡	31½	32½
29	6⅙	⅓	5⅙	7½	94	11⅙	17⅔
7	7	1	0	0	0	0	0
25	7	2 7/13	*	Under 15	29	Under 15	Under 15
11	7½	4¾	17	23	34	17	34
6	7½	14	29½	36	33½	28½	—
27	7½	2	12½	20¾	53½	14½	24½
20	8	3	21	*	0	21	66
28	8⅙	1 11/13	17⅚	34	68	*	42
18	8¼	2⅔	20	25	0	0	0
4	8⅓	2½	*	18½	0	23½	33½
19	8 5/13	1	15	35	62	17	60
14	8½	1¼	*	18½	0	17	—
24	8½	1 11/13	14 7/13	38	‡	*	53
1	9¼	4¼	61½	*	98½	74½	—
9	9⅔	8	25	35	†	20⅔	—
15	9¾	1½	16	34	0	14	49
16	10	6	28	38	‡	28	—
30	12⅙	2¼	12 11/13	23¾	32	25¾	28¾
5	13⅓	4	16	16	60+	14	16
21	14	3	0	0	0	0	0

* Recurred; exact time not noted.

† Insufficient time.

‡ Did not return under observation.

EXPLANATION OF PLATES.

Plate XLIX.

Fig. 1. Microphotograph of a left dorso-lateral segment of the spinal cord from the cervical region. Stained by the Marchi method. The degeneration is almost entirely localized to the fasciculus lateralis pyramidalis and the fasciculus lateralis proprius (Flechsigi). The splotch in the gray matter is a negative defect. From Experiment 27; puppy eight months old; death by chloroform; resuscitation after 7 minutes and 30 seconds; return to an apparently normal condition in about three weeks; and examination after another week. (Zeiss Planar 2, B. & L. oc. 2.)

Fig. 2. Microphotograph of the fasciculus lateralis pyramidalis from the same section as I with higher magnification. (Experiment 27; Marchi method; Zeiss obj. A. B. & L. oc. 2.)

Plate L.

Fig. 3. Microphotograph showing adjoining portions of the pyramids of the medulla, also from the same case as Figs. 1 and 2. Outside of these, there is only an occasional degenerated fiber, and higher sections traced the degeneration to the motor cortex. (Zeiss obj. A. B. & L. oc. 2.)

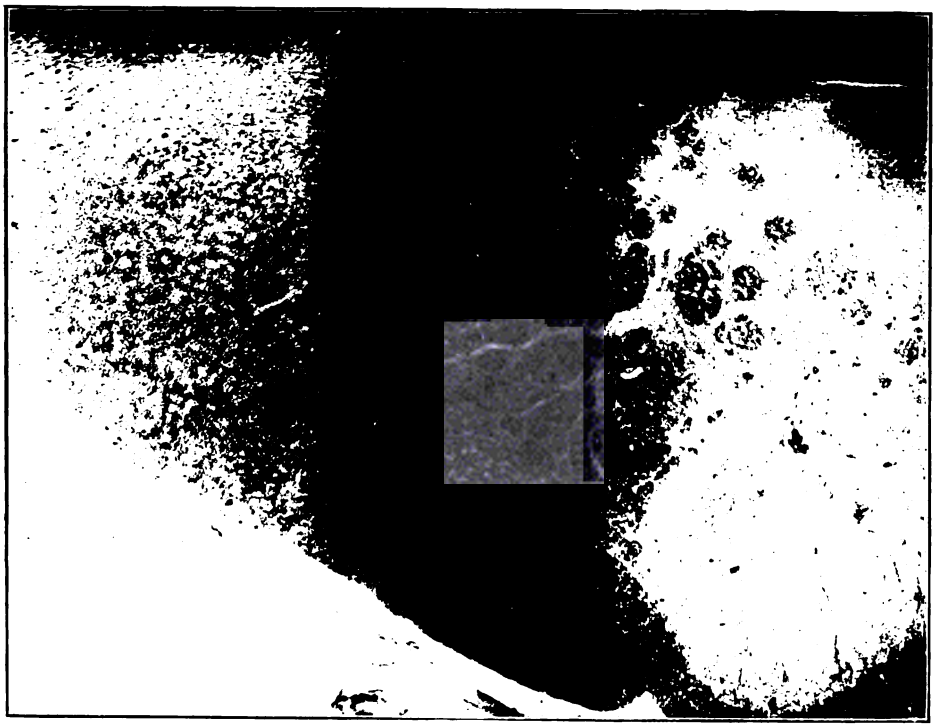

FIG. 1.

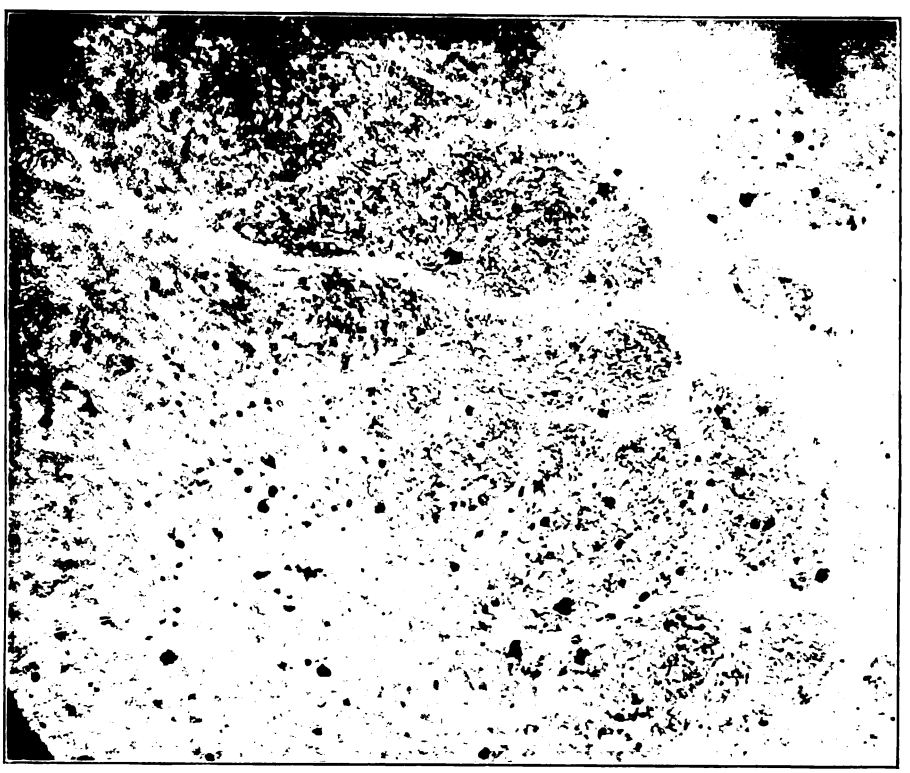

FIG. 2.

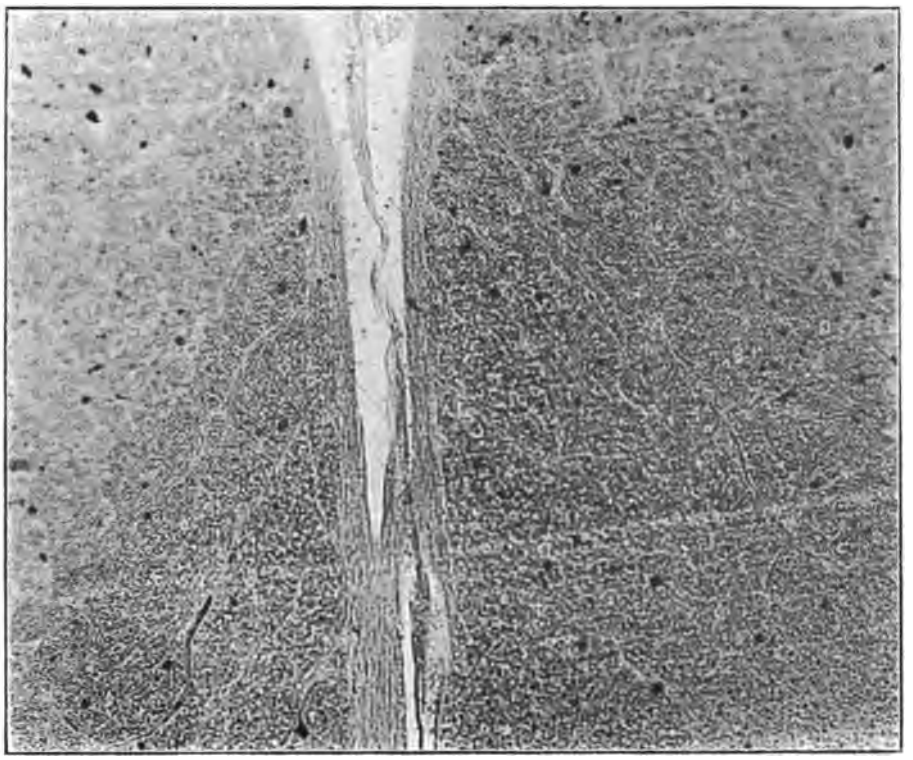

FIG. 3.

THE REACTIVE CELL PROLIFERATION IN THE WHITE RAT; AND ITS RELATION TO THE GENESIS OF TRANSPLANTABLE TUMORS.[1]

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There is hardly any doubt left in the minds of pathologists that the transplantable tumors in the white rats and mice, recently discovered by Hanau (1), L. Loeb (2), Jensen (3)and others, resemble clinically very closely human carcinoma and sarcoma. These tumors grow to large size, occasionally weighing as much as the animal on which they grow; they are malignant to the animal, and frequently fatal to the host. There were noticed local recurrences after extirpation of the original tumor and metastases, usually in the lungs.

The main feature distinguishing the tumors of white rats and mice from human cancer as well as from tumors of most other animals, lies in the fact that the latter cannot be transplanted into another individual of the same species, while in the former this can be accomplished not infrequently. The transplantability of these tumors may be due to the great inherent power of proliferation of the tumor cells. This inherent proliferating capacity would then be, not only much greater than in any normal cell, but also greater than the proliferating power of a cancer cell of any other animal species. This power would not depend on the character of the tissues to which the cell is transplanted.

On the other hand, it is possible that the cancer cell in a white rat or mouse is not different from a cancer cell in any other animal species, but the organisms of the former animals may react differently to tumor transplantations.

[1] Read by title at the eighth annual meeting of the American Association of Pathologists and Bacterologists, in April, 1908. Received for publication June 23, 1908.

If one accepts the first factor as the only one controlling the transplantability of the mice and rat tumors, then it would be logical to regard transplanted tumors as artificially produced metastasis. On the basis of that assumption one could not expect much information regarding the genesis of tumors from the experimental transplantation of tumors from animal to animal. But there are a number of facts in the experimental research of these transplantable tumors which seem to indicate very strongly that cellular, and possibly also metabolic reactions of the host, contribute a great deal to the success of the transplantation of these tumors. The powers of limitless proliferation ought to be equally inherent in every cancer cell, no matter to what animal species it belongs. A human cancer cell transferred anywhere through the lymph or blood circulation will proliferate and form a metastasis very frequently of a much larger size than the original tumor. But it is impossible to make this cell proliferate when transferred to any other organism. In the innumerable operations done for cancer, surgeons have most probably often enough cut themselves, and thus become inoculated with some cancer cells, and yet there is not one report to the effect that one was in this way infected with cancer. The cancer cell of the white mouse or rat *per se* is certainly not different from any other cancer cell, in as much as a tumor cell of a white mouse while growing readily when implanted in another mouse, grows slightly only when implanted in another animal, in a white rat for instance, a species which is very near the white mouse, and imperfectly even when implanted in a white mouse of a different race. Very interesting in this connection is the so-called zigzag-transplantation of Ehrlich and Apolant (4). One transplants a tumor of a white mouse into a white rat. The tumor grows only for a while and then ceases. A few days later he takes the tumor from the rat and implants it another mouse, where the tumor grows strongly. He then removes the tumor from the mouse and implants it again into a rat and the growth ceases after a while, and so on. Ehrlich (5), who is of the opinion that cancer growth is due to the proliferating power of the cancer cell, admits that for the growth of a mouse tumor there is required the addition of an X substance which exists in the mouse and is absent in the rat or any other animal.

The work of Gaylord and Clowes (6), Ehrlich (5), Michaelis (7), Bashford (8), Flexner and Jobling (9) seems to indicate that it is possible either to increase or to decrease artificially the success of the tumor transplantation in a white mouse or rat, produce a so-called "athreptic" immunity. Ehrlich calls this phenomenon "athrepsia" because he thinks that the difference in the success of a transplantation is due to the difference in the nutrition that the implanted cell receives in the new soil.

All these facts indicate very clearly that the success of tumor transplantation in the white mouse or rat is due to the difference in the reaction of the tissues to the implantation of cancer cells as compared with other animals or man.

While cancer cells are different from normal cells in their power of unlimited proliferation, this power manifests itself only when a certain specific stimulus is added by the host to the implanted cancer cells. More than that, some facts in the recent research with the transplantable tumors seem to indicate that the growth of a transplanted tumor is not necessarily due to the proliferation of the implanted cancer cells, stimulated to it by some factor derived from the host. In some cases the reverse may be the case and the growth of a tumor may be due to the proliferation of the normal cells of the host, stimulated to it by some factor derived from the implanted cancer cells. According to J. Orth (10), even human cancer may in some cases grow in this way. As he states it, there are cancers in which the transformation of preformed epithelial cells into cancer cells takes place.

C. Lewin (11) recently reported a series of experiments in which he transplanted human carcinoma of the uterine cervix into white rats. The implanted piece became necrotic and around it there developed a growth of granulation tissue. He succeeded in re-implanting these granulomata into two generations. In none of his tumors could he detect any microörganisms either by staining or by cultivation.

While different mechanical and chemical injuries may produce a granuloma, cancer tissue, in Lewin's opinion, acts differently inasmuch as it produces a transplantable granuloma. It is self-evident that in this case the stimulus acts on the cells of the host.

Quite similar to Lewin's results, seem to be the reported cases of transformation of a transplanted carcinoma into a sarcoma in subsequent generations. Apolant (12), L. Loeb (13), Bashford Murray and Haaland (14) have all reported such cases, and they all lately give practically the same explanation to the phenomenon. Through a specific stimulus (Reiz) produced by the implanted carcinoma cells, a new connective tissue stroma is formed by the host. this stroma in subsequent generations becomes ever richer in cells, and, finally, these cells of the host under the influence of something emanating from the implanted carcinoma cells become sarcomatous and transplantable. It is clear, then, that the cellular activity of the host contributes something to the success of transplantation of the tumors of the white mice and rats.

It seemed desirable to investigate whether the reactive cell proliferation of these animals will also be different from other animals, when the stimulus to this proliferation is given, not by an implantation of cancer tissue, but by a different agent.

While the transplantable tumors of the white mouse are usually carcinomata developing from the mammary gland, most of the tumors of the rat reported by Velich (15), Flexner and Jobling (16), Loeb (17), and Herzog (18) are sarcomata and of all the immense number of rat tumors examined there are only two cases of carcinoma reported, Hanau (19), Michaelis and C. Lewin (20).

It is always easier to produce a proliferation in cells of the connective tissue type, and consequently this investigation was conducted on white rats.

I reported previously the results of experiments with implantation of normal tissue (21) and also with introduction of aleuronat in the form of an emulsion or triturate tablets (22). The objectionable feature of aleuronat consists in the fact that it is a substance producing an inflammation and the formation of pus. There has lately been described an agent acting more directly on the proliferating power of the local fixed cell, without the aid of migrating leucocytes. In 1907, Fischer (23) reported a series of experiments in which he injected subcutaneously in a rabbit's ear, oil containing scharlach R, a fat-staining substance. He produced in this way an extensive irregular proliferation of epithelial

cells, giving the morphological appearance of an epithelioma. His results were corroborated by Jores (24), Snow (25) and Helmholz (26), who found besides the epithelial cells some proliferation of connective tissue cells also.

I used this substance on white rats in the following variety of methods:

I. Subcutaneous Injection of Scharlach R in Paraffin.—A saturated solution of scharlach R in soft paraffin was employed, and the usual surgical technique followed. Three weeks after the injection the paraffin with the tissue surrounding it was excised. In every experiment the paraffin was surrounded with a thick connective tissue wall from which chords were running in the paraffin. Both the wall and the chords consisted mainly of young round and spindle cells, with very little fibrous tissue.

II. Subcutaneous Injection of Scharlach R in Oil.—For these experiments a sterile saturated solution of scharlach R in oil was used. Five injections were made in the same place in every animal at intervals of forty-eight hours. Three days to two weeks after the last injection the animals were killed. There always developed in the place of the injection a cyst which contained in its center some unabsorbed oil. The walls consisted of the subcutaneous fat completely impregnated with young round and spindle cells, the latter frequently combined in regular rows.

III. Injection of Scharlach R-Oil in the Mammary Gland.—The same oil used for Experiments II was injected into the mammary gland through the nipple. Five injections were made in the same gland at intervals of forty-eight hours. Two weeks after the last injection the animals were killed. The glands were always found greatly enlarged and containing some of the oil in the center. Microscopically no change in the glandular tissue was detected, but the connective tissue was filled with round and spindle cells. The microscopical picture certainly resembled a great deal more a sarcoma of the breast than a chronic mastitis.

IV. Transplantation of Scharlach R-Mammary Glands.—Small pieces of the mammary glands of the animals from Experiment III were transplanted subcutaneously or in the peritoneum. Ten days after the transplantations the animals were killed. The

implanted pieces were always found somewhat enlarged. The peritoneal pieces were usually attached to the omentum. Microscopically the implanted pieces appeared necrotic and surrounded by a thick layer of round and spindle connective tissue cells. The cells appeared to grow into the implanted piece.

Dr. J. H. Larkin assisted me in the preparation of the microscopical specimens and I take great pleasure in extending my thanks to him.

The analysis of these experiments shows that the white rat reacts with a profuse connective tissue cell proliferation to different stimuli, whether it is implantation of normal rat tissue or human cancer tissue, whether an injection of aleuronat or scharlach R solutions. The latter substance produces in a rabbit mainly an epithelial proliferation, but in a white rat, no matter how or where employed, it acts only on connective tissue cells.

That it is impossible microscopically to determine in every instance whether we are dealing with a sarcoma or a granuloma, can be seen from the studies with the transplantable sarcoma in the dog. Sticker (27) and Ewing and Beebe (28) maintain that it is a sarcoma, while Apolant (29) and Bashford, Murray and Cramer (30) are inclined to consider it an infectious granuloma. Ehrlich considers it a microscopical proof of sarcoma when there are regular intersecting rows of spindle cells. But the same picture can be found in the formations resulting from the injection of scharlach R.

Ehrlich claims further that the sarcoma which developed in his case from carcinoma grew too fast for an ordinary granuloma. But the cysts developing after injection of aleuronat or scharlach R grow just as rapidly and may also attain a very large size.

After transplantation of pieces of scharlach R mammary glands, the new connective tissue cell formations were not as extensive nor as malignant to the host as after implantation of pieces of rat tumor. But the difference is apparently only quantitative. Not every sarcoma of a white rat is transplantable, and when it is, the weight of evidence seems to tend toward the explanation that in every new host it is the cell of the host that forms the new growth under stimulation by the implanted piece. There can cer-

tainly be no qualitative difference between the implantation of normal rat tissue or pieces of mammary gland that have previously been subjected to the influence of scharlach R, on the one hand, and the implantation of human cancer tissue, on the other. Still C. Lewin reports the development of malignant transplantable granuloma after implantation of a piece of human cancer in a white rat, which, by the way, took place only in two animals from a whole series of experiments.

It is interesting to note here that while all the pieces of normal tissue (skin, liver, spleen, testicle or mammary gland), when transplanted under the skin or in the peritoneum, retain their cellular structure, the transplanted pieces of scharlach R-mammary gland in the same length of time (ten days after implantation) become necrotic. This necrosis is not due to the injection of scharlach R, as the similar pieces of mammary gland without transplantation show no signs of necrosis. The same central necrosis took place in the pieces of human carcinoma reported by C. Lewin and also usually takes place in the retransplanted pieces of sarcoma of the white rat. Apparently the peripheral new cellular formation takes place more rapidly in transplantation of cancer tissue or of tissue subjected previously to the influence of scharlach R, than around normal tissue, and consequently the former is sooner cut off from the food supply and sooner becomes necrotic. In a transplanted sarcoma at least it is impossible to prove definitely that the new growth in the host is due to the proliferation of the implanted cells and not to the proliferation of the cells of the host, and the implanted piece acts only as a stimulant. It is not so easy to indicate the influence of the reactive power of the host in transplantation of carcinoma, but even there some facts point in the same direction. Apolant reports on a transplanted carcinoma which on a subsequent transplantation was transformed into adenoma. C. Lewin reports a case of carcinoma in a white rat which was transformed in subsequent generations into an adenoma, then again into a carcinoma, then into a sarcoma. Loeb saw a sarcoma change into an endothelioma. All such mutations of the morphological appearance of the tumors are very difficult to explain on the supposition that the growth of a transplanted tumor is due only to the

proliferation of the transplanted cells without the reactive proliferation of the cells of the host. This extensive reactive cellular activity of the organisms of the white rat and mouse is the most important factor in the modern biological investigation of the genesis of cancer and will contribute mainly to the elucidation of its problems.

As Bashford, Murray and Haaland state it, in connection with their report where an implanted carcinoma changed into a sarcoma, this cellular activity of the host puts us in a position to follow the formation of the actual malignant tumor from its very incipiency. On the other hand, if the growth of a transplanted tumor is due only to the proliferation of its own cells, then these tumors represent only metastasis, and the study of them could only elucidate the course of one of the later phases in the development of tumors.

The work reported here is still in progress and conditions may still be found under which a malignant transplantable tumor will be produced artificially in white rats or mice.

In conclusion I deem it a pleasant duty to express here my gratitude to Dr. T. Mitchell Prudden for the privilege of the laboratory and for the interest shown in my work.

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FIG. 1.

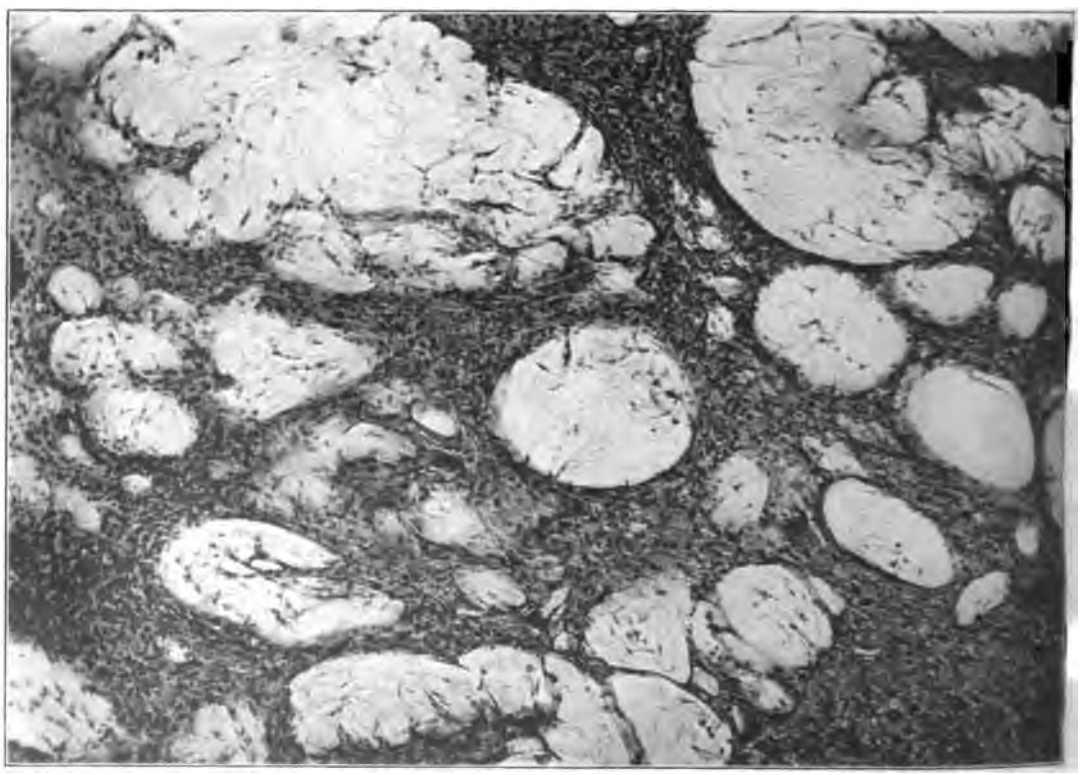

FIG. 2.

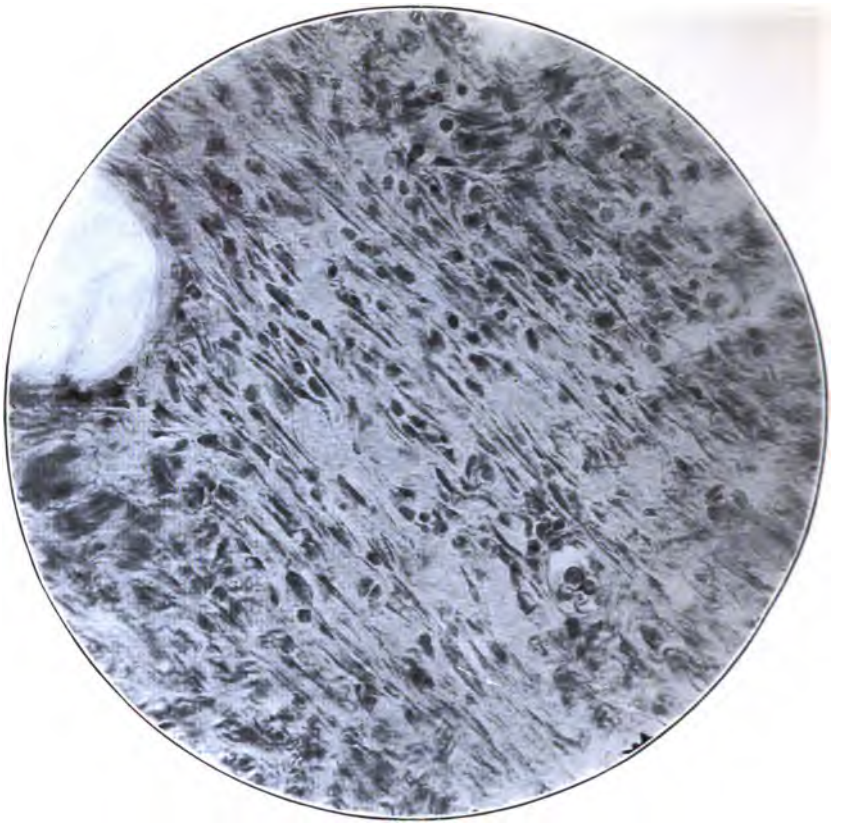

FIG. 3.

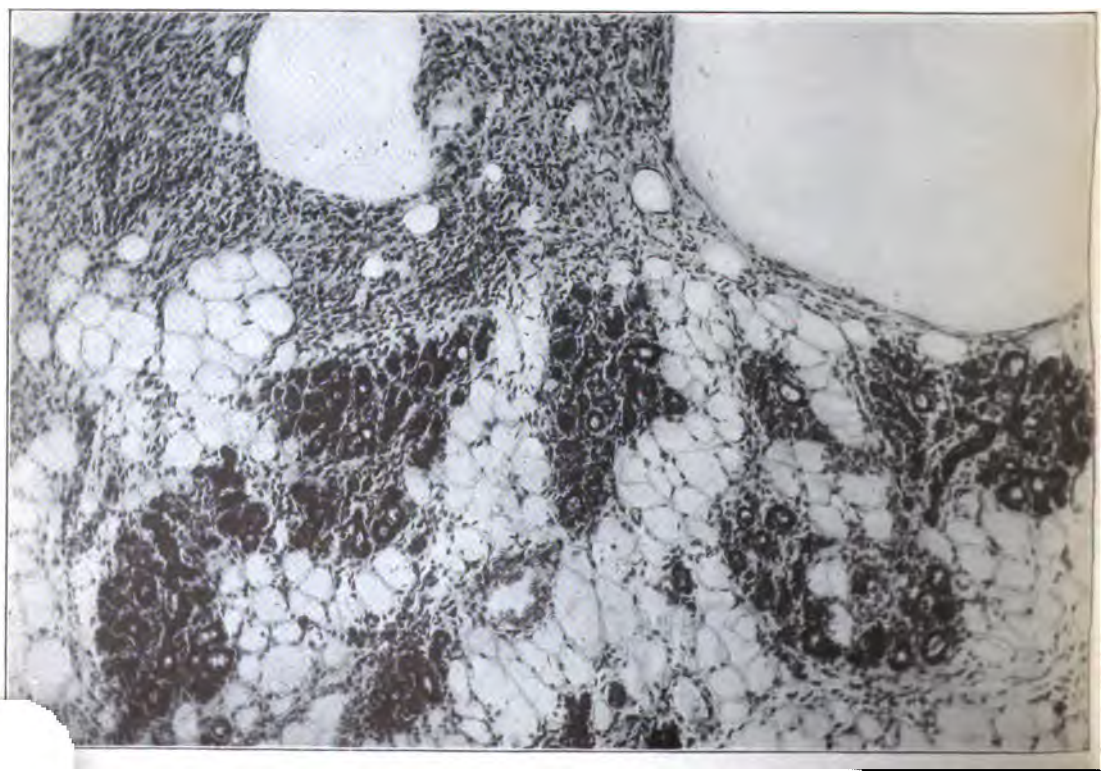

FIG. 5.

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EXPLANATION OF PLATES.

Plate LI.

Fig. 1. Scharlach R-paraffin cyst. Low power shows part of the wall and the net of chords.

Fig. 2. Same as Fig. 1, but higher power; shows the round and spindle cells.

Plate LII.

Fig. 3. Scharlach R-oil cyst. Subcutaneous fat impregnated with round and spindle cells.

Fig. 4. Mammary gland treated with scharlach R in oil. The connective tissue filled with round and spindle cells.

Plate LIII.

Fig. 5. A piece of mammary gland treated with scharlach R in oil and then transplanted into the peritoneum of another rat. Specimen shows the necrotic implanted piece surrounded by a layer of round and spindle cells.

SOME FACTORS IN THE PHYSIOLOGY AND PATHOLOGY OF GASTRIC SECRETION.[1]

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The following studies were commenced nearly three years ago, the end in view at that time being to ascertain, if possible, the effect induced on gastric function by a simple, mechanical narrowing of the pyloric orifice. In the course of numerous experiments to determine certain limits and variations of normal secretion, new questions arose, the solution of each of which appeared interesting in itself or necessary as a preliminary to the furtherance of the original plan. Some of the data collected in these observations will be presented in this paper.

These experiments were conducted on dogs on which the Pawlow operation had been done. This operation in our hands has been modified in no essential particular except that it has been found necessary to unite with special care the muscle layer forming the division between the two stomachs. When this is not done the flaps of mucous membrane used to cover this wall do not have sufficient strength and a communication between the two stomachs results, necessitating a second operation.

The manner of conducting an observation was simple. The dog was suspended from a frame by means of a canvas hammock in which were holes for the legs and a small opening at the orifice of the fistula. By means of a flanged rubber cannula the escaping gastric juice was collected in small graduates. In a hammock such as is here described the dogs can be left for a number of hours without discomfort, indeed they usually sleep unless disturbed.[2]

[1] This work has been aided by a grant from the Rockefeller Institute for Medical Research. Received for publication July 13, 1908.

[2] In conducting investigations of this character on dogs too much emphasis cannot be placed upon the necessity of feeding a uniform diet regularly. It is

The Pawlow school has devoted much attention to the subject of the various types of gastric secretion that are excited by meat, bread, or milk. It is particularly interesting to note that in the rare cases of gastric fistula in human beings[3] the conceptions of Pawlow and his pupils have received a confirmation which leaves no doubt that the results of such experiments are strictly analogous to similar conditions in man. This fact has a special significance not only for experimental physiology, but also in the study of pathological gastric conditions, since in the latter we must ascertain the effects of single isolated factors before full comprehension of a morbid function is possible. The effects of such factors are only to be derived from experiments on animals. This phase of the subject will be given further consideration in another part of this paper.

From an *a priori* standpoint it has long been taught that large amounts of water should not be taken with meals, the idea being that much water dilutes the salivary and gastric juice and in consequence impairs their activities. On the other hand it has been argued that one of the functions of gastric juice is to bring the chyme to a proper degree of fluidity through the "*Verdünnungs Sekretion*" of von Mering. Pawlow[4] has shown experimentally that water stimulates the flow of gastric juice but that this is true only when comparatively large amounts are ingested, *e. g.*, 400 to 500 c.c. Small amounts, *e. g.*, 100 to 150 c.c., excite little or no result. The

desirable, in order to obtain large volumes of gastric juice, that the animals take daily liberal amounts of fluid; but to place a vessel of water in the cage will not answer this purpose, because uniform amounts of fluid are not taken voluntarily each day. The proper amount of water (40 c.c. per kilo of body weight is our standard) must be mixed with the food for the dog. In this way the dog is given sufficient fluid and the amount not left to the animal's caprice. The importance of this metabolism standard becomes evident if varying amounts of fluid are intentionally given for a few days and the results on the secretion of gastric juice after the same test meal are noted. When there is abundant fluid in the body tissues, the secretion is copious; but if the body tissues cannot spare much fluid, the gastric secretion is scanty. The importance of this precaution is evident in studies of this nature. It is not unlikely that this fact has therapeutic significance in those gastric disorders in humans wherein the gastric secretion is subnormal in amount.

[3] Cade and Latarjet, *Compt. rend. Soc. Biol.*, 1904, lvii, 496. Hornberg, Inaug. Dissertation, Helsingfors, 1903. Umber, *Berlin. klin. Woch.*, 1905, xlii, 56.

[4] Pawlow: The Work of the Digestive Glands. Trans. by Thompson, London, 1902.

same conclusions had previously been advanced, however, by Heidenhain[5] and by Sanozky.[6] Pawlow's special contribution was his demonstration that the secretion which is stimulated by water persists even after the vagi are divided, a proof that such stimulation is not nervous but chemical in character. So far as we know there is no record of experiments to determine the effects upon secretion of varying amounts of water, taken with food. Pawlow intimates his appreciation of the fact that water in any quantity may be a source of error in testing the effect of a substance upon the gastric glands, but he has described no experiments marking the degrees and limitations of such effects.

Our attention was first directed to the influence of water upon the amount of gastric secretion by the results of our endeavors to regulate the diet of dogs having Pawlow double stomachs so that there would be a minimum of erosion of the skin about the orifice of the fistula. It was repeatedly noticed that when the food given to these dogs was mixed with but little water, the epithelium grew to some extent over the eroded surface; when much water was given with the food, however, the wound on the next day appeared raw and inflamed. These results led us to collect the amounts of juice secreted after meals containing widely different amounts of water.

We found that water acts as a stimulant to gastric secretion when it is taken mixed with the food; the degree of stimulation effected by water is in proportion to the amount imbibed; and, so far as we have been able to note, this law is not influenced by the nature of the accompanying food. Our observations have been made usually with either cracker meal or hashed raw meat mixed with the water, and, for either of these alone or definite proportions of the two, the amount of gastric secretion was largely influenced by the amount of water given with them. Tables I and II illustrate this point.

The question at once arises: What are the limitations to this increased activity which is excited by water? With amounts of water less than 200 c.c. the variations, if any, are slight and not constant There is obvious difficulty in administering more than 700 c.c. of

[5] Heidenhain, *Archiv. f. d. gesam. Physiol.*, 1879, xix, 148.
[6] Sanozky, *Arch. d. sci. biol.*, 1892, i, 589.

TABLE I.

Food: 200 grams of cracker meal.

Hour.	300 c.c. Water.		500 c.c. Water.		750 c.c. Water.	
	Hourly Amount of Juice, c.c.	Total Amount of Juice, c.c.	Hourly Amount of Juice, c c.	Total Amount of Juice, c.c.	Hourly Amount of Juice, c.c.	Total Amount of Juice, c.c.
1	1.8		2.7		6.0	
2	1.0		2.6		5.7	
3	1.7		4.9		6.5	
4	1.8		6.3		5.9	
5	0.9	7.2	1.2	17.7	1.6	25.7

TABLE II.

Food: 300 grams of meat.

Hour.	No Water.		500 c.c. Water.	
	Hourly Amount of Juice, c.c.	Total Amount of Juice, c. c.	Hourly Amount of Juice, c.c.	Total Amount of Juice, c.c.
1	3.7		9.8	
2	3.0		9.6	
3	1.4		9.7	
4	2.1		8.0	
5	2.0		5.5	
6	1.0		2.4	
7	0.0	13.9	1.3	46.3

water to a dog of ordinary size and approximately that quantity could be given only by using smaller amounts of food material more diluted, *e. g.*, 150 grm. meat with 600 c.c. water, but in these experiments no further response could be excited in the gastric glands. The reason for this may be that the food matter settles out from the suspension in the stomach when the amount of water is relatively large, in the same manner as in a beaker, the superabundant water passing on through the pylorus.

This influence of water was brought out in even a more striking manner in another set of experiments in which milk was used as a test food.

In the endeavor to find various methods for testing the accuracy of our results, milk in different degrees of concentration was given the dogs as food. Since milk can be kept without souring for several days, it offered an absolutely uniform food substance for several control observations. If the facts already mentioned rela-

tive to the influence of water on gastric secretion are correct, then milk with reduced water content should when fed call forth less secretion than natural milk. This was found to be the case. When 500 c.c. of milk were fed, the secretion during the first two hours amounted to about 20 c.c., but after the same volume of milk was concentrated to half its bulk by evaporation at a low temperature,

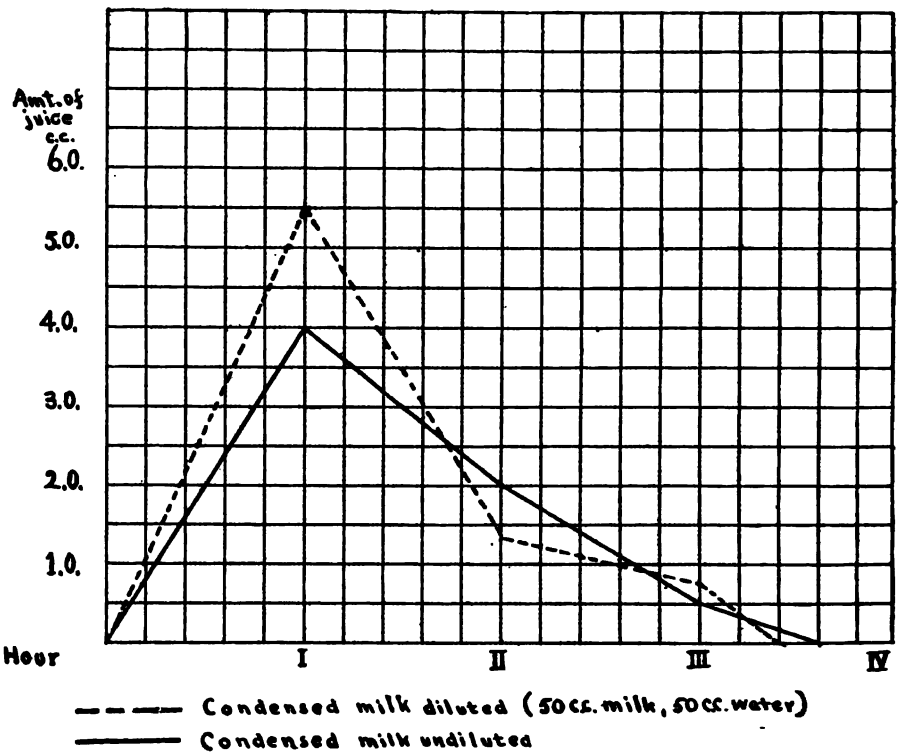
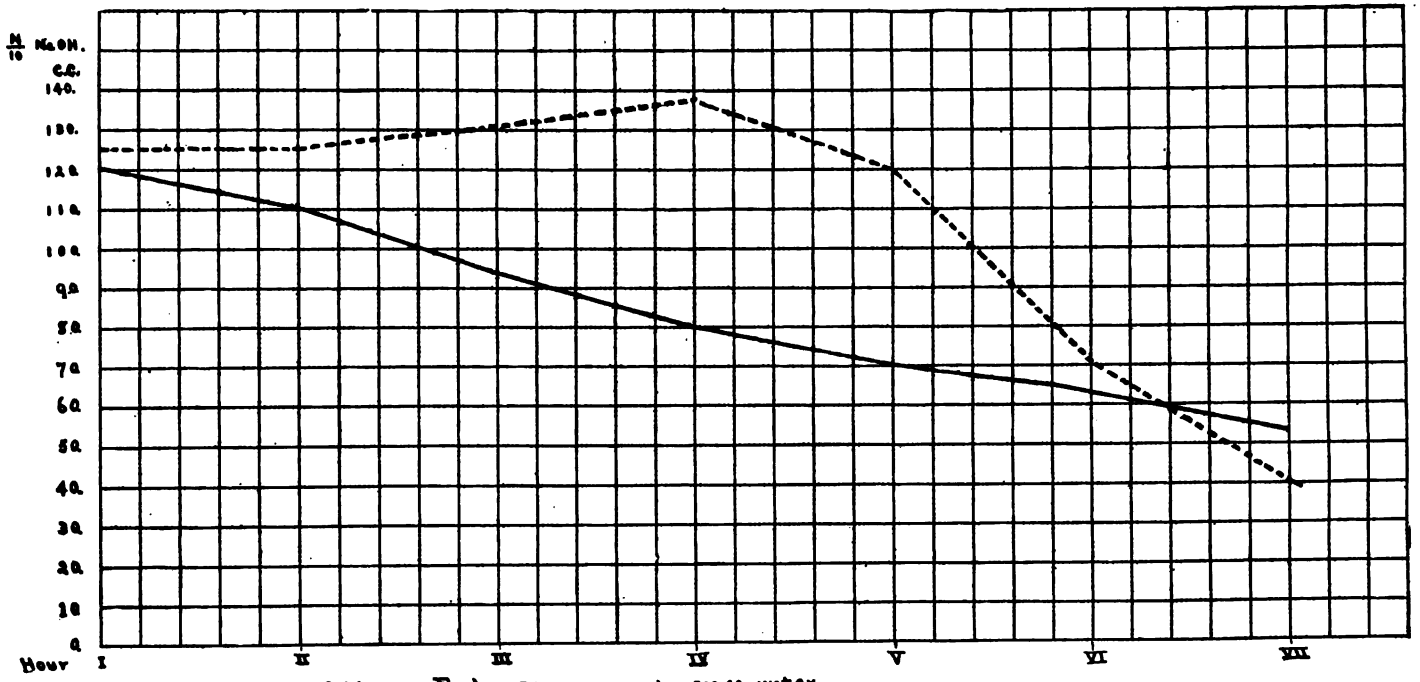


CHART I.

the secretion excited by this condensed product was only about four fifths of that induced in the first case.

Since it is conceivable that this result was due in part at least to changes effected by heat in the condensation of the milk, we ascertained the comparative effect of feeding condensed milk diluted to its original volume. For this purpose use was made of milk condensed at a low temperature to half its original volume. When such



Acidity Food:- 300 grams meat 500 c.c. water.

" " 300 " " no water.

Acidity recorded in c.c. of N/10 NaOH require to neutralize 100 c.c gastric juice

CHART II.

a condensed milk was fed, the secretion that resulted was relatively slight in amount. When the diluted product was given, however, the secretion was appreciably augmented. The accompanying chart (Chart I) shows the curves of secretion, for condensed milk alone and for the product restored to its original volume.

When the secretion of the gastric glands is abundant the acidity is much higher than it is when the secretion is scanty. This is shown in the accompanying chart (II) where comparative curves are plotted.

Water causes therefore not only a more voluminous secretion but also a more acid secretion. We have found no facts that serve as a complete explanation of this phenomenon. Digestion proceeds at an optimum when there is a certain acid concentration and, if water be taken copiously with food, more acid and hence, more juice must be secreted to bring the chyme to the proper acid concentration. That the gastric glands have a considerable range of adaptability to digestive requirements is known,[7] and this may be held to be an instance of such adaptability.

If this explanation is correct, and there appears some foundation for it in the acid curves then it is possible that the generally accepted idea, that copious drinking of water with food is injurious, may have a physiological basis. Under such conditions the glands must compensate for the diluted state of the chyme by excessive secretion, and since glandular activity probably requires relatively as much energy as any other form of activity, this special secretion is a form of extravagance. We have endeavored to find out if increased demands, such as are exemplified under these conditions, influence the activity of the glands at the next feeding. We are convinced that in some degree they do; for when dogs are fed with 100 grams of meat and relatively large amounts of water and then after five or six hours are again fed, the second meal excites under these conditions less gastric juice than is ordinarily the case when little or no water is added to the initial meal. This suggests a sort of gland fatigue.

In explanation of the increased secretion occasioned by ingestion of water, it is possible that the hormone which Edkins[8] styles gas-

[7] Pawlow, *loc. cit.*

[8] Edkins, *Journal of Physiology*, 1906, xxxiv, 133.

tric secretin may play a rôle, in that water may promote the formation or absorption of this hormone. But while it seems not improbable in the light of what is known of pancreatic secretin that such a hormone is a factor in the activity of the gastric glands, the methods employed by Edkins in his experiments are not above criticism and leave much to be desired for the elucidation of the problem he sought to solve.

On examining the tables showing degrees of gastric secretion, it may be seen that the volume of juice during the first hour is much increased when there is considerable water in the food. Since this initial flow is, in great part, what Pawlow names the "appetite juice," its occurrence here may be fully explained by the fact that water increases the bulk of the food mass and in the act of feeding the animal receives a more prolonged taste sensation (and perhaps more intense also) than when the food is small in bulk and can be quickly devoured. That is to say, the added water increases the nervous stimulus to the gastric glands through the sense of taste. This initial active juice would, in case proteins were present, bring about a rapid digestion to proteoses and peptones, substances which act as chemical stimuli to the gastric glands and in this manner increase secretion in the second stage of digestion. This explains why it is that the maximum flow of gastric juice is late when dogs are fed on cracker meal which has relatively little taste and excites a scanty appetite juice and hence a relatively slow production of the chemical excitants, proteose and peptone.

THE ACIDITY OF GASTRIC JUICE.

When the gastric juice is collected in hourly periods following a test meal, the secretions of the small stomach are observed to vary considerably in acidity. Not only is this true of the hydrochloric acid that is present as free acid but also of the total acid as evidenced by titration with tenth normal sodium hydroxide in the presence of phenolthalin. Ketscher,[9] working in Pawlow's laboratory, noted that when the juice flows rapidly, the acidity is higher than it is when the hourly secretion is sparse and his explanation is that the mucus neutralizes the hydrochloric acid. If the secretion is

[9] Pawlow, *loc. cit.*

copious it flows more rapidly over the alkaline mucous membrane and the acidity under these circumstances is higher, according to Ketscher, than when the flow is slow and opportunity for neutralization is better.

Pawlow concluded from Ketscher's experiments that the gastric juice is equally strongly acid whether it be rapidly or slowly poured out. In so far as Ketscher's explanation applies to variations in the amounts of *free* hydrochloric acid in the secretion it is doubtless correct. The variations in the amounts of free acid are marked and the quantities of free acid are lowest, as estimated by titration, when mucus is most abundant. It has been shown that mucus has the power of combining with hydrochloric acid.[10]

If the statement of Pawlow relative to constancy of acid is correct, the gastric secretion would form an exception to present ideas of glandular activity. In the case of all other secretions there is a considerable variation in the composition of the product put out by the glands under varying conditions, and in the stomach Pawlow admits the fluctuations of peptic power. It is rather difficult to understand why Pawlow comes to his conclusion regarding constancy of acidity. While it is true that the decreased flow of secretion gives more opportunity for neutralization of the acid by mucus, this result would produce no appreciable effect on the total acidity when that acidity is estimated by titration with sodium hydroxide in the presence of phenolthalin, because the sodium cation would replace the mucus in the mucus-hydrochloric acid compound since a strong base always replaces a weaker one. With this chemical fact in mind it became impossible for us to understand Pawlow's contention in the face of titrimetric results.

In order to ascertain whether there is a fluctuation in the hydrochloric acid content of the juice or a uniform acidity of secretion, a careful estimation of the chlorides became necessary. As a preliminary to the use of the Volhard method for chlorine determination, a measured amount of unfiltered gastric juice was neutralized with sodium hydroxide and evaporated to dryness in a porcelain crucible. This residue, after slightly charring over a low flame, was extracted with hot water, and the ash collected on an ashless

[10] Foster, *American Jour. of Med. Sciences*, 1907, cxxxiii, 303.

filter paper which was returned to the crucible and again charred. This procedure was carried through three times before final incineration in order not to lose chlorides by volatilization. The filtrates were collected, made up to a definite volume with water and the chlorin estimated as usual by the Volhard method. When the incineration is made with gastric juice not previously neutralized with sodium hydroxide the hydrochloric acid that exists as free acid or in combination with protein is broken up and volatilized. The resulting estimation will give the chlorine present as salt.

The method above described has been used constantly as a check on the titrimetric results and it has shown that there is a variation in the amount of hydrochloric acid secreted by the gastric glands. This variation, while not great, is considerable and indicates that the gastric secretion is not different from other glandular secretions in the matter of fluctuating quantitative composition. The percentage content of hydrochloric acid in gastric juice fluctuates from hour to hour. Early in the digestive period the content of chlorine in the juice is higher than toward the end of digestion. In the accompanying table are shown the differences for consecutive hours in two typical experiments.

TABLE III.

Dog VIII.

Hour.	Total Chloride.[11] Per Cent. HCl.	Salts. Per Cent. HCl.	Volatile Chloride. Per Cent. HCl.	Secretion Per Hour. c.c.
1	0.547	0.146	0.401	5.0
2	0.621	0.073	0.548	6.2
		Dog XIV.		
1	0.672	0.110	0.562	15.4
2	0.626	0.101	0.525	11.0
3	0.523	0.085	0.438	5.8
4	0.367	0.057	0.310	1.8
5	0.237	0.018	0.219	0.6

These observations raise the question: Within what limits does the hydrochloric acid content of the gastric juice vary? In attempting to answer this question several factors require consideration, and first among them is the individual. Two dogs seldom respond

[11] After neutralization with sodium hydroxide.

to the same food by secreting juices of identical character. As might be expected the absolute quantity of secretion from the small stomach will depend upon its size to some extent but, irrespective of the amount of secretion, the percentage content of acid is different in the gastric juice from different dogs fed on the same kind of food. A second factor has been clearly brought out by Pawlow, that is, that each food substance excites a type of secretion which, in some degree, is peculiar to that food. For example, the amounts of secretion per hour following a meal of bread or of milk are different from those excited by meat. With these two considerations in mind it becomes evident that limitations of variability must be computed for each animal experimented upon and the only idea of limitation of function that we can gain must depend upon an average computed from the data for a number of animals.

TABLE IV.

Dog.	Food.	Hour of Digestive Period.	Acidity.		
			High Per Cent.	Low Per Cent.	Difference. Per Cent.
II.	Meat	1	0.401		
II.	"	6		0.237	0.164
III.	Cracker	1	0.456		
III.	"	4		0.329	0.127
IV.	"	1	0.438		
IV.	"	4		0.255	0.183
VI.	Meat	1	0.438		
VI.	"	3		0.328	0.110
VII.	"	1	0.565		
VII.	"	4		0.245	0.320

From these estimations it would seem that there is a variation in percentage content of hydrochloric acid in gastric juice at different times during digestion. The amount of variation while not large is, however, considerable, amounting to a difference of from 0.1 to 0.3 per cent., during the digestive period.

EFFECT OF ORGANIC ACIDS ON GASTRIC SECRETION.

Two years ago Berg and Gies completed, in this laboratory, the first phase of a study of peptic digestion in dilute solutions of various common organic and inorganic acids and mixtures of acids, in vitro. Among their most striking results were the observations that pepsin

is comparatively powerless to bring about proteolysis in dilute solutions of acetic acid (unless very much pepsin is present), but, on the other hand, that acetic acid, even when present in a comparatively large proportion, does not appreciably affect the proteolytic efficiency of relatively very small amounts of pepsin in dilute hydrochloric acid. In other words, under the ordinary conditions of gastric digestion, acetic acid taken into the stomach as vinegar neither helps nor harms, appreciably, the purely chemical process of peptic proteolysis.[12]

These and related observations led Berg and Gies to state their intention of studying possible secretory effects of acetic acid and vinegar in the stomach.[13] At Gies's suggestion, however, we undertook this study together with an investigation of the gastro-secretory effects of various other organic acids that are used extensively as condiments or are taken in beverages, or which occur in the stomach as products of fermentation.[14]

The following acids have been used in this study: acetic, citric, lactic, and butyric. The method of administration was the same in all the experiments. 100 c.c. of 0.5 per cent. solutions of acid were used instead of an equivalent volume of water mixed with the food.

Under pathological conditions lactic acid is the most common of the organic acids to appear in the stomach as a result of bacterial activity. It is of special interest, therefore, to learn what effect this acid has upon secretion and upon digestion.

Lönnqvist[15] studied the effects of lactic acid and butyric acid by giving them unmixed with food to dogs with Pawlow double stomachs. Lönnqvist's observations show that these acids, under the conditions mentioned, have more stimulating action upon the gastric mucous membrane than the same amounts of water. When these acids were given with food, however, in our experiments, we failed to note any evidence of stimulated secretion.

[12] Berg and Gies, *Proc. of the Society for Exper. Biol. and Med.*, 1906, iv, 17; *Journal of Biological Chemistry*, 1907, ii, 489.

[13] Berg and Gies, *Journal of Biological Chemistry*, 1907, ii, 534 and 545.

[14] Dr. Berg meanwhile has proceeded here with a study, soon to be published, of the comparative digestibility in vitro of many proteins in given solutions of a certain number of common acids.

[15] Lönnqvist, *Scandinavisch. Archiv f. Physiol.*, 1906, xviii, 194.

TABLE V.

Food: 100 grams hashed meat. Fluid 100 c.c.

Hour.	100 c.c. Water. c.c. Juice.	100 c.c. 0.5 Per Cent. Lactic Acid. c.c. Juice.	100 c.c. Water. c.c. Juice.	100 c.c. 0.5 Per Cent. Butyric Acid. c.c. Juice.
1	11.2	10.0	9.6	9.7
2	5.0	5.4	2.6	3.2
3	1.6	1.6	1.8	2.6
Totals.	17.8	17.0	14.0	15.5

These observations have been repeated a sufficient number of times to convince us of the constancy of the results, not only with these acids in 0.5 per cent. solution, but in 0.1 per cent. concentration also.

It is a popular opinion that vinegar taken with food aids in some way in the digestive process. This might be due to an increase in the amount of appetite secretion excited by the condiment, or there might be in vinegar some substance which acts as a chemical stimulant to the gastric glands. Both vinegar and lemon juice are common popular remedies for slight disorders of digestion and in some diseases, notably chlorosis, the appetite for acids of this nature is pronounced.

In our experiments we have employed both vinegar and lemon juice; with the first the results have not been concordant. At times vinegar apparently caused an increase of secretion during the first two hours of the digestive period. In other cases there has been no increase over the control. The vinegar employed contained 4.8 per cent. acetic acid and 10 c.c. were given with the food in every case. That the particular substance in vinegar which possibly acts as a stimulus might be determined, experiments were conducted with acetic acid and with the distillate from vinegar which had been neutralized before distillation. Acetic acid in no case caused any increased secretion. The distillate from vinegar acted in the same way as vinegar, that is, the results were erratic. Why this is so it is not possible to say since the dogs were healthy and there was no disparity in the controls. The increase of secretion when it occurs after taking vinegar is during the first two hours of the digestive period, which would indicate that the increase is due to a

more copious output of "appetite" juice, that is, that any influence which this condiment exercises is due rather to the flavor it imparts to food than to any stimulating effect upon the glands in the chemical sense.

TABLE VI.

Dog XII. Food: 100 grams hashed meat.

Hour.	100 c.c. Water. Juice c.c.	90 c.c. Water, 10 c.c. Vinegar. Juice c.c.	100 c.c. Vinegar Distillate. Juice c.c.	90 c.c. Water, 10 c.c. Vinegar. Juice c.c.	100 c.c. Vinegar Distillate. Juice c.c.
1	9.0	8.4	7.0	10.9	10.4
2	7.1	7.9	5.3	8.2	6.9
3	4.5	4.0	1.1	3.6	4.1
Totals	20.6	20.3	13.4	22.7	21.4

Neither lemon juice nor citric acid appear to have the property of increasing the flow of gastric juice. From this negative evidence one must conclude that if the acids have any therapeutic value, it is due to their effect upon the organic metabolism and not upon a stimulation of gastric secretion.

TABLE VII.

Dog X. Food: 100 grams hashed meat.

Hour.	100 c.c. Water. c.c. Juice.	100 c.c. 0.5 Per Cent. Citric Acid. c.c. Juice.	100 c.c. 0.5 Per Cent. Acetic Acid. c.c. Juice.	90 c.c. Water. 10 c.c. Lemon Juice. c.c. Juice.	90 c.c. Water. 10 c.c. Vinegar. c.c. Juice.
1	11.00	9.9	11.1	10.5	9.5
2	5.8	4.9	6.1	6.0	5.0
3	1.6	1.7	1.5	2.0	1.8
Totals.	18.4	16.5	18.7	18.5	16.3

The above results in vivo are in accord with those obtained in vitro by Berg and Gies, in that these organic acids neither increase nor retard the digestion process, but act simply as inert substances when taken into the stomach with food. No emphasis can be placed upon the effects due to vinegar and the vinegar distillate since the results are not constant and uniform.

EFFECT ON SECRETION OF STENOSIS OF THE PYLORUS.

The clinical study of morbid gastric function, while it may have defined certain symptom complexes as associated more or less inti-

mately with apparent changes in gastric efficiency, has nevertheless not developed any clear ideas of cause and effect in the pathological physiology of gastric disease. In conditions of disease, whatever may be the primary cause, the picture finally presented to the clinician is a symptom complex. In the case of functional diseases of the stomach, the effect produced on secretion by any possible cause operating alone is, at present, hardly a matter of scientific knowledge, as the prevailing indefinite ideas concerning the physiology of hyperchlorhydria amply prove. The normal physiology of gastric secretion and motility, as we understand it to-day, is so complex and correlated with that of the duodenal mucous membrane,[16] the bile, and pancreatic juice, that no clear-cut conception of cause of morbid function is possible until single isolated factors have been fully and successfully studied experimentally, and the kinds and degrees of their effects recorded.

A consideration of the normal physiology of gastric function suggests that pathological conditions in the stomach may take origin in the duodenum as well as in the stomach itself. Obstruction of the normal flow of bile delays the neutralization of the first portions of acid material ejected from the stomach,[17] and this acid, acting upon the duodenum, prevents the relaxation of the pyloric sphincter in its normal manner, since it is known that acid in the duodenum effects a tonic closure of the pylorus.[18] The same delay in gastric evacuation would be induced by any factor which inhibited the formation or absorption of secretin. The complexity of the problem presented in any abnormal state is quite evident. Moreover, as Cannon has pointed out, there is in every disturbance of the normal relations a readjustment of functions to meet the new demands and this readjustment obscures the etiological factors in the case.

In clinical laboratories occasional researches have been made for the purpose of determining the effects of single factors, such for example as obstruction of the flow of bile into the intestine as a cause of hyperchlorhydria.[19] But the limitation put upon clinical

[16] Cannon, *Amer. Jour. of Physiol.*, 1907, xx, 283.

[17] Cannon, *loc. cit.*

[18] Hirsch, *Cent. f. klin. Med.*, 1893, xiv, 73 and 377. Serdinkoff, Inaug.-Diss., St. Petersburg, 1899. Reviewed in *Hermann's Jahresbericht*, 1899, viii, 204–14.

[19] Riegel, *Zeitsch. f. klin. Med.*, 1886, xi, 167; Simnitzky, *Berlin. klin. Woch.*, 1901, xxxviii, 1077.

research in such relations by the inability to secure pure gastric juice, or to measure the amount of secretion with any degree of accuracy, unfortunately impairs the value of the results obtained.

An experimental study of the effects produced on the rate of discharge of chyme from the stomach, by ligating the bile and pancreatic ducts, has been conducted by Cannon.[20] Under these conditions there is considerable retardation of the passage of food into the duodenum as compared with that under normal circumstances. In the case of Cannon's experiment the effect then results from a temporary stenosis of the pylorus, due positively to the action of the hydrochloric acid upon the duodenal mucosa and negatively to the absence of the normal alkaline salts which neutralize the acid. The experiment is significant in suggesting one of many ways by which the gastric function may be influenced by extrinsic causes.

It is to be supposed that the secretory as well as the motor function would be altered in some manner by the prolonged retention of food in the stomach. This would appear the more probable if there is a hormone which brings about a chemical stimulus to the gastric glands, for such conditions would favor the production and absorption of gastric secretin. The work of Rubow[21] is here suggestive. Cases of apparent hyperacidity are often due, according to this author, to the collection of gastric juice in a stomach, which, on account of loss of muscle tone or impediment at the pylorus to the exit of food, is unable to empty itself normally.

It appears desirable, therefore, in order to gain an insight into the relationship of the more complex factors, to learn the effects on gastric secretion of a simple mechanical interference with the passage of chyme from stomach to duodenum.

Lönnqvist[22] made some observations in this connection, but the conditions of his experiments were so artificial that trustworthy deductions from them cannot be made. In Lönnqvist's dog the duodenum was divided at the pylorus, and the end of the intestine and the pyloric end of the stomach fastened into the abdominal wall, forming two fistulæ, a connection between which could be made at

[20] Loc. cit., p. 303.
[21] Rubow, *Archiv f. Verdauungs-Krankheiten*, 1907, xiii, 577.
[22] Lönnqvist, *loc. cit.*

will by means of a tube. In this experiment the time of discharge from the stomach was easily regulated through the connecting tube. Under such conditions, however, many factors other than the simple arrest of normal discharge enter into and complicate the problem. For this reason a method has been employed in our work that produced the minimum disturbance of relations consistent with the attainment of a marked degree of obstruction at the pylorus.

After the operation of making the Pawlow double stomach, sufficient time was allowed for the dog to recover normal health and for us to conduct a number of control observations on secretion that would serve as a normal base line for future use. Then, at a second operation, a band[28] made of silver wire was placed around the pylorus and pressed down snugly upon a stomach tube passed through the mouth of the dog into the duodenum. This tube served as a guide to the size of the pyloric opening that was left.

It was found by preliminary experiments that this sort of band about the pylorus would in a few days become imbedded in newly formed connective tissue and that, the peritoneum overgrowing this new formed tissue, the final result was an annular cicatrix about the pylorus.

After a period of a month following the operation of making the double stomach, the second operation above mentioned was accomplished on March 2, 1908 (Dog XIII). The animal quickly recovered from the anesthetic and six hours after operation appeared as well as usual. The day following operation, the dog was fed her regular meal of meat, cracker meal, lard and water. This meal was vomited in the course of two hours after eating. Consequently, since the dog appeared hungry, several small meals were given during the course of the day (March 3). The same procedure was repeated on the second and third days after operation. Even large amounts of water induced emesis while repeated smaller quantities of 100 to 200 c.c. were retained. The irritability of the stomach disappeared gradually and after two weeks, feeding of the larger food masses was resumed and retained. Excepting a moderate loss in weight following the operation, the health of this dog remained good for

[28] The band was made of silver wire by weaving two strands into a coarse mesh half an inch in breadth and two inches long.

some time. She ate her food greedily each day and was active. Early in April, however, there was a rather sudden change; she began to lose weight and strength. The appetite was poor, and continued so, and when it became evident that the animal could not be nursed to health, she was killed with chloroform.

Observations were commenced on March 7, five days after the second operation. The effect of the stenosis on gastric secretion is made evident by comparing the results obtained before and after stenosis. The figures below are representative of many observations.

TABLE VIII.

100 Grams Hashed Meat and 100 c.c. Water.								50 Grams of Cracker Meal and 100 c.c Water.	
Before Stenosis.				After Stenosis.				Before Stenosis.	After Stenosis.
Hour of Digestion	Juice c.c.	Juice c c.	Average c.c.	Juice c.c.	Juice c.c.	Juice c c.	Average c.c.	Juice c.c.	Juice c.c.
1	18.5	20.2	19.3	6.9	8.7	8.1	7.9	5.3	4.6
2	16.1	15.3	15.7	9.2	7.1	9.8	8.7	5.5	4.5
3	12.6	10.3	11.4	9.3	6.9	9.0	8.4	3.3	3.5
4	5.8	6.0	5.9	7.9	5.1	4.6	5.8	2.0	1.5
5	1.1	1.2	1.1	7.0	6.5	2.2	5.2	—	1.7
6				4.0	4.9	4.0	4.3	—	2.0
7				2.0	3.5	2.1	2.5	—	1.8

On examining this table two facts at once attract notice: the prolongation of the digestive period and the decrease in the amounts of hourly secretion. The prolongation of the digestive period, to some extent at least, is what might be expected in view of the mechanical obstruction to the outflow of chyme into the intestine. Even a high grade of hypertrophy of the musculature of the stomach could hardly compensate for the degree of stenosis here effected.[24] Since the products of protein digestion which act as chemical stimulants to secretion (proteoses and peptones) would be formed and their delayed removal from the stomach would perhaps favor their greater activity as secretogogues, the lengthened period of activity of the gastric glands after a given meal appears to be a natural result. Moreover the diminution in the hourly

[24] At autopsy a glass rod 6.3 mm. in diameter could barely be passed through the plyoric lumen.

amount of secretion would result in the necessity for more time to effect the disintegration and acidulation of the food mass.

It could readily be shown that the prolongation of secretion was due primarily to the special retention of food in the stomach and not to other factors, by feeding substances which are of such a nature that the pyloric obstruction becomes inoperative; for example, peptonized milk. When thoroughly peptonized milk was fed, the digestive period appeared to be finished in about three hours. If the same volume of unpeptonized milk were fed, however, the digestive process lasted nearly five hours. In normal dogs peptonized milk calls forth only a scanty flow of gastric juice during the first hour after feeding.

TABLE IX.

Hour of Digestion.	200 c.c. Milk. Juice c.c.	200 c.c. Peptonized Milk. Juice c.c.	200 c.c. Peptonized Milk + HCl.[88] Juice c.c.	Normal Dog. 200 c.c. Peptonized Milk. Juice c.c.
1	7.8	8.1	7.5	2.4
2	7.4	7.6	7.5	0.2
3	5.1	1.0	2.1	
4	3.2			
5	1.8			

In case the pylorus still exercised its normal function on the conditions of this experiment, the diminished secretion would nevertheless bring about a delay in the appearance of free hydrochloric acid and hence retard evacuation. The addition of acid sufficient to saturate the proteins should shorten under these conditions the digestive period. This was the object in some experiments in feeding peptonized milk to which acid had been added. There was, however, no evident effect from the acid administered in this way.

The second notable result of the stenosis was the diminution in the amount of hourly secretion of gastric juice. This diminution was confined to the first three hours of digestion. Subsequent to the fourth hour, the amount of secretion was considerably greater *after* stenosis of the pylorus than normally. The chart (Chart III) illustrates this point. The digestive period following a meal of 100 grams of meat was usually completed in about five hours, whereas

[88] Hydrochloric acid was added to milk until all protein was acid-bound as determined by reaction with Günzberg's reagent.

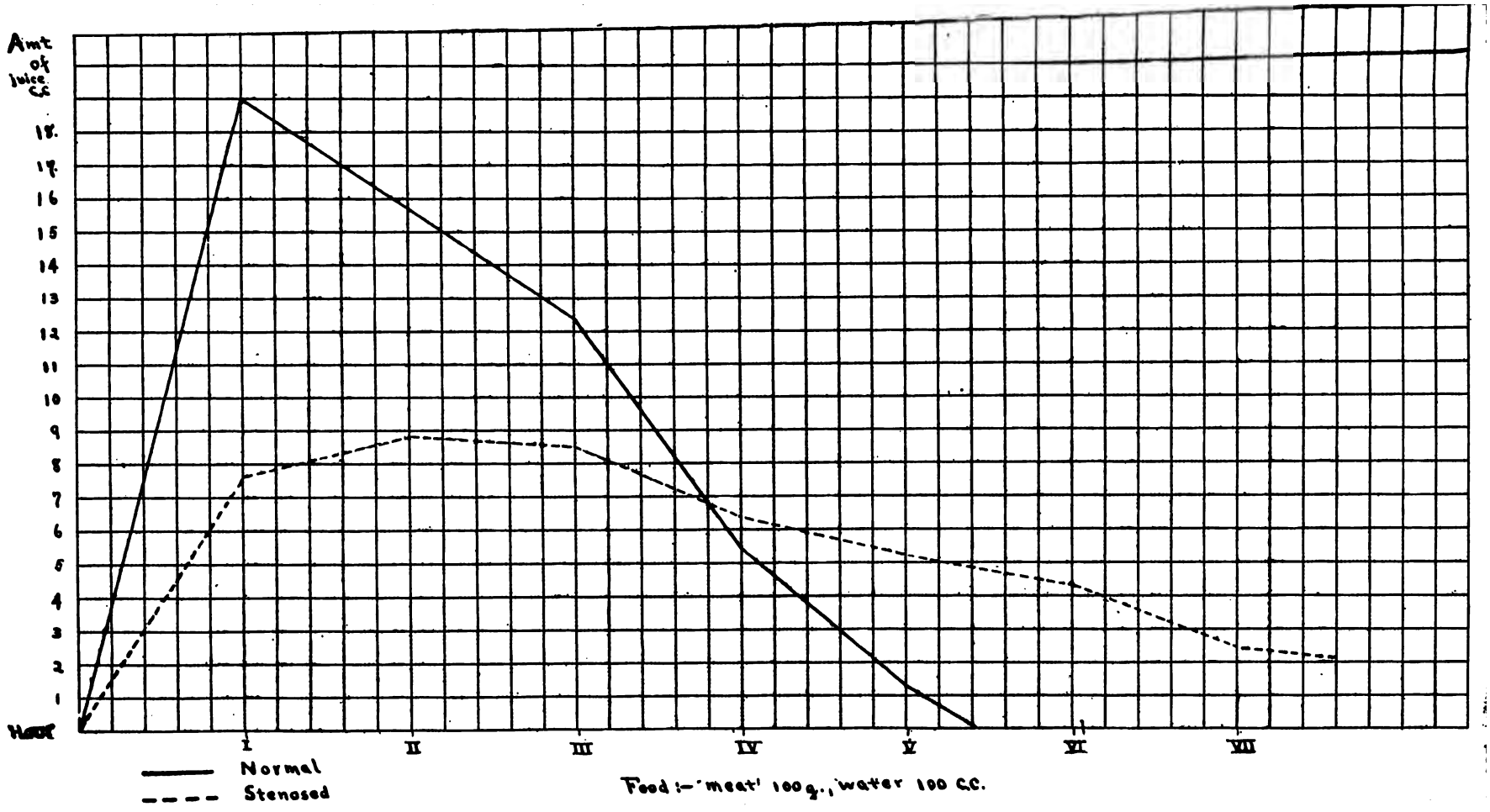

Amt of Juice c.c.
18.
17.
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Hour
I
II
III
IV
V
VI
VII
Normal
Stenosed
Food:- meat 100 g., water 100 c.c.

CHART III.

in dogs with stenosed pylori, there was still active secretion in the sixth and seventh hours. We shall have occasion to revert to this fact later in considering constant secretion.

It has been shown that the amount of gastric juice in the first hours of digestion is due, in great part, to the psychic or appetite influence[26] and, since this is the time when the effect of mechanical obstruction at the pylorus made itself most manifest in our dogs by diminishing the amount of juice secreted, the only deduction that seemed possible was that in some manner the nervous control of the gastric glands was affected by the operation. That the degree of

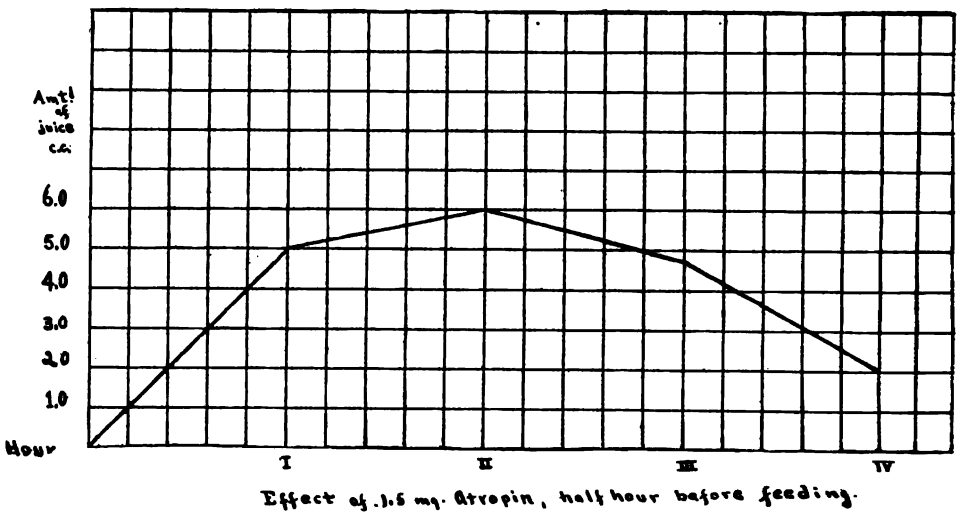


CHART IV.

nerve stimulus, as represented by appetite and taste sense, was the same after operation as before, there is no evidence further than that the dog ate greedily and with apparent relish. An injury to nerve filaments through pressure of the silver band is conceivable but speculative.

In order to test the correctness of this explanation that the reduced amount of secretion was in effect a juice resulting from a chemical stimulus rather than a psychic one, atropin was used. When atropin is administered hypodermatically to a normal dog an half hour before the animal is fed, there is no secretion of gastric

[26] Pawlow, *loc. cit.*, pp. 80–92.

juice until over two hours after feeding—the atropin completely inhibits the activity of the secreting cells. When secretion finally commences under such conditions the juice cannot be appetite or psychic juice, for the dog long since finished eating, and in our own experiments, usually was asleep in the observation hammock. Any secretion under such conditions must be the result of chemical stimulus. The curves (Chart IV) showing rate of gastric secretion in normal dogs after administration of atropin bear out the first supposition that, in the dogs with stenosis, the slow secretion of juice is due chiefly to chemical stimulation rather than to an influence of the psychic centers acting through nerve control.

After the second operation, that of producing the stenosis, it was noted that there was an appreciable, constant secretion of gastric juice. The construction of the artificial stomach was particularly well adapted for the detection of juice secreted after the digestive periods, since the fistulous end of the pouch had been drawn through the split fibers of the rectus muscle and attached to the skin so that the muscle acted as a valve preventing the escape of nearly all secretion. Consequently when a cannula was inserted into the small stomach the accumulated juice could be drained off. It was first noted that after stenosis there was a considerable amount of normal gastric juice in the small stomach (Dog XIII) each morning, whereas, in other dogs, and in this dog before the stenosis had been made, the small stomach contained at most a few drops of mucus. It was supposed that this secretion was the result of food material remaining in the stomach, and gastric lavage was instituted at night without, however, effecting the result expected. In order to get a clearer idea of what the conditions were, lavage was performed ten hours after feeding and, commencing a few hours later, hourly collections of the secretion of the small stomach were made. Under these conditions it was clearly evident that there was a constant secretion of juice from the small stomach amounting to 1.5 to 2.0 c.c. per hour. These samples contained much mucus and in this respect, as in that of acidity, resembled the secretion at the end of a period of digestion. The acidity averaged about 0.14 per cent. This condition of constant secretion continued unabated until the animal was killed. It was quite as pronounced during the period when the

dog appeared active and in health as later when she was evidently sick.[27] Since the continued secretion bore no relation to food residue in the stomach, and, so far as we were able to judge, was not influenced by the general condition of the dog, one must conclude that the obstruction at the pylorus was the cause of this secretion.

The more important points considered above in relation to Dog XIII were confirmed by instituting the same procedure with another dog. Our studies were, however, interrupted by a sickness of the latter dog which led us to kill her with chloroform.

At the autopsies done upon control dogs with stenosis as well as on those with Pawlow double stomachs, wherein stenosis of the pylorus was produced, there was noted in every case considerable dilatation of the stomach. This dilatation was most marked in the cardiac third of the organ. In the dogs with Pawlow stomachs that had been kept alive for several months after the operation for constricting the pylorus, there was observed, in addition to the dilatation of the stomach, a marked grade of hypertrophy of the muscle coats in the region of the antrum. The mucous membrane was not altered in its macroscopic or microscopic appearance, thickening of the stomach walls being due entirely to an increase of the muscle layers. The dilatation in these dogs was not pronounced, not so marked in fact as in the case of the control dogs which were killed a few days after the pylorus had been constricted.

ADDENDUM.

Tables Supplementing those in the Text.

Dog VII. Food: 200 grams cracker meal.

Hour.	300 c.c. Water. Hourly Amount Juice, c.c.	300 c.c. Water. Hourly Amount Juice, c.c.	500 c.c. Water. Hourly Amount Juice, c.c.	750 c.c. Water. Hourly Amount Juice, c.c.	1,000 c.c. Water. Hourly Amount Juice, c.c.
1	0.3	1.4	2.7	5.9	3.5
2	1.0	2.0	2.6	5.7	2.3
3	1.7	1.7	4.9	6.5	1.8
4	0.4	1.0	4.0	5.0	0.9
Totals	3.4	6.1	14.2	23.1	8.5

[27] Dr. Edward Cussler, while working in this laboratory in 1907, had a dog that presented this same condition. It was found subsequently at autopsy that in the operation of making the double stomach the first incision had been made so close to the pylorus that the resulting scar had effected an obstruction of the lumen. Dr. Cussler's study has not been published.

Dog X.

Hour.	Food: 500 c.c. Milk.		Food: 500 c.c. Milk Condensed to 250 c.c.		Food: Condensed Milk, 50 Grams.	
	Hourly Amount Juice, c.c.	Hourly Amount Juice, c.c.	Hourly Amount Juice, c.c.	Hourly Amount Juice, c.c.	Hourly Amount Juice, c.c.	+50 c.c. Water. Hourly Amount Juice, c.c.
1	2.8	2.7	5.0	4.8	2.1	3.4
2	7.8	9.0	6.2	6.0	1.8	2.3
3	6.4	8.7	1.2	1.8	.1	.4
Total.	17.2	20.4	12.4	12.6	4.0	6.1

Dog XI.

Hour.	Food: 50 Grams Bread.		Food: 200 Grams Meat and 100 Grams Cracker Meal.	
	50 c.c. Water. Hourly Amount Juice, c.c.	100 c.c. Water. Hourly Amount Juice, c.c.	200 c.c. Water. Hourly Amount Juice, c.c.	300 c.c. Water. Hourly Amount Juice, c.c.
1	7.5	8.6		
2	5.4	7.3		
3	3.9	6.0		
Totals	16.8	21.9	24.0	30.1

Dog III.

Hour.	Food: 300 Grams Meat.		Food: 200 Grams Cracker Meal.		
	No Water. Hourly Amount Juice, c.c.	500 c.c. Water. Hourly Amount Juice, c.c.	300 c.c. Water. Hourly Amount Juice, c.c.	300 c.c. Water. Hourly Amount Juice, c.c.	750 c.c. Water. Hourly Amount Juice, c.c.
1	3.7	9.8	2.7	2.8	3.2
2	3.	9.6	1.6	2.6	
3	1.6	2.8	1.2	2.0	7.1 (2 and 3) hour
Totals	8.3	22.2	5.5	7.4	10.3

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Date Due

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